Intervention levels for the support of blood pressure in extremely preterm infants

by

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Abstract

Background

The true relationship between different cardiovascular variables are complex. A varied practice exists in the cardiovascular management of extremely preterm infants, including intervention levels for blood pressure (BP). Adverse outcomes may be secondary to low BP, anti-hypotensive treatment, or both.

Aim

To test the hypothesis that alterations in BP and cardiac output (CO) have an effect on cerebral blood flow (CBF), that these cardiovascular measures effect electroencephalographic (EEG) continuity and that different BP intervention levels will result in different rates of inotrope usage and achieved levels of BP in infants born <29 weeks gestation using patients recruited to a clinical pilot study.

Methods

Infants had measurements of CO and CBF using ultrasound, EEG recording and BP profiles downloaded for the first postnatal week. Infants were randomised to different levels of mean arterial BP at which they received cardiovascular support: Active(<30mmHg), Moderate(< Gestational age mmHg) or Permissive(signs of poor perfusion or <19mmHg). Once this BP threshold was breached, all infants were managed using the same treatment guideline. Cranial ultrasound scans were reviewed blind to study allocation. The relationship between physiological measurements was explored. The validity and reliability of

CBF measurements were examined using flow phantom models.

Results

Sixty infants were recruited, had detailed measurements performed, and randomly assigned to one of three arms. CO was not related to CBF or BP. Inotrope usage, and invasively measured BP on day 1 were highest in the Active and lowest in the Permissive arm. There were no differences in haemodynamic or EEG parameters, or non-cerebral clinical complications. EEG continuity and CBF were directly related to BP. The validity and reliability of CBF was acceptable. Composite rates of grade 2–4 intraventricular haemorrhage, periventricular leucomalacia or parenchymal cysts were significantly different on post-hoc analysis between the three arms (Active 0/19, Moderate 6/20, Permissive 2/21;p=0.014).

Conclusion

Alterations in BP and CO did not affect CBF. Different BP intervention levels resulted in different rates of inotrope usage and levels of achieved BP. Although EEG continuity was not different between the three arms, this study found an increasing mean arterial BP was associated with increasing cerebral electrical activity and higher EEG continuity.

This thesis is dedicated to Priyanka, Joshua and my parents

for their unconditional love and support.

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Abbreviations Used

aEEG	Amplitude integrated encephalogram
ANOVA	Analysis of variance
BP	Blood pressure
BAPM	British Association of Perinatal Medicine
BPD	Bronchopulmonary dysplasia
CO	Cardiac output
CRF	Case report forms
CV	Coefficient of variation
CCA	Common carotid artery
CCAF	Common carotid artery blood flow
CI	Confidence interval
CrUSS	Cranial ultrasound scan
d	diameter of right common carotid artery
DMC	Data monitoring committee
db	decibels
D	Diameter of the aortic valve
EEG	Electroencephalogram
cFTOE	Cerebral fractional tissue oxygen extraction
FiO2	Fraction of inspired oxygen
GE	General Electric
HDU	High dependency unit

HR	Heart rate
Hz	Hertz
IBI	Interburst interval
IVH	Intraventricular haemorrhage
ICC	Intraclass correlation
IWM	Intensity weighted mean
IWMV	Intensity weighted mean velocity
ln	Natural log
LoA	Limits of agreement
LV	Left ventricle
LVO	Left ventricular output
MABP	Mean arterial blood pressure
MHz	Mega hertz
ml/kg/min	millilitres per kilogram per minute
ml	millilitres
ml/min	millilitres per minute
mmHg	millimetres of mercury
MRI	Magnetic resonance imaging
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
NIRS	Near infra red spectrometry
NRES	National research ethics service
OR	Odds ratio
PAC	Pulmonary artery catheter
$PaCO_2$	Partial pressure of arterial carbon dioxide

PaO_2	Partial pressure of arterial oxygen
PDA	Patent ductus arteriosus
PRF	Pulse repetition frequency
PTFE	Polytetrafluoroethylene
PVH	Periventricular haemorrhage
PVL	Periventricular leukomalacia
RCCA	Right common carotid artery
RCCAF	Right common carotid artery blood flow
rcSO2	Regional cerebral oxygen saturation
REC	Research ethics committee
ROP	Retinopathy of prematurity
RVO	Right ventricular output
SCBU	Special care baby unit
SD	Standard deviation
SVC	Superior vena cava
TSH	Thyroid stimulating hormone
T4	Thyroxine
VTI	Velocity time integral
Xe	Xenon
π	Pi

Statement of originality

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Publications, Presentations and Awards

Publications in peer reviewed journals

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Pereira SS, Kempley ST, Wertheim DF, Sinha AK, Morris JK and Shah DK (2018) Investigation of EEG Activity Compared with Mean Arterial Blood Pressure in Extremely Preterm Infants. Front. Neurol. 9:87. doi: 10.3389/fneur.2018.00087

Abstract publications

SS Pereira, C Jones, DK Shah, D Wertheim, S Kempley. *Relationship between EEG and continuously measured invasive blood pressure in extremely preterm infants in the first three days of life*. Arch Dis Child Fetal Neonatal Ed 2014; 99: Suppl 1 A77-A79doi:10.1136/ archdischild-2014-306576.221

SS Pereira, AK Sinha, DK Shah, ST Kempley. *Relationship between carotid blood flow, cardiac output and blood pressure in extremely preterm infants*. Arch Dis Child 2014;99:A147 doi:10.1136/ archdischild-2014-307384.397

C Jones, **SS Pereira**, D Wertheim, DK Shah, S Kempley. *Variability of blood pressure*

in extremely preterm infants in the first 72 hrs of postnatal adaptation. Arch Dis Child Fetal Neonatal Ed 2014;99:Suppl 1 A53-A54 doi:10.1136/ archdischild-2014-306576.152

Presentations

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SS Pereira, AK Sinha, DK Shah, ST Kempley. *Relationship between carotid blood flow, cardiac output and blood pressure in extremely preterm infants*. European Academy of Paediatric Societies, Barcelona, 2014 and The Neonatal Society, London, 2013.

Pereira SS, Kempley ST, Wertheim D, Sinha AK, Shah DK. *Continuous invasive blood pressure is directly related to EEG measure of continuity in extremely preterm infants in the first three days of life*. The Neonatal Society, London, 2013

Poster presentations

Pereira SS, Sinha AK, Shah DK, Kempley ST. *Variation Between Staff Recorded and Continuously Downloaded Invasive Blood Pressure Measurements in Extremely Preterm*

Infants. Pediatric Academic Societies Meeting, Baltimore, Maryland, 2016.

Pereira SS, Sinha AK, Shah DK, Wertheim DF, Morris J, Kempley ST. *Intervention levels for the support of blood pressure in extremely preterm infants: A pilot randomized trial*. Pediatric Academic Societies Meeting, Baltimore, Maryland, 2016.

SS Pereira, C Jones, DK Shah, D Wertheim, S Kempley. *Relationship between EEG and continuously measured invasive blood pressure in extremely preterm infants in the first three days of life*. Pediatric Academic Societies Meeting, San Diego, California, 2015.

Awards

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Contents

List of Figures

List of Tables

Chapter 1

Introduction and Background

1.1 Introduction

Neonatal intensive care has witnessed significant progress over the last few decades which has led to an improved survival of extremely premature infants (Wilson-Costello 2007, Costeloe et al. 2012, Johnson and Marlow 2017). An increase in the survival of infants born at the threshold of viability (Figure 1.1) is attributed to improved care, centralisation of services and the use of antenatal steroids (Lasswell et al. 2010, Travers et al. 2017). Despite considerable progress being made in the overall care given to these infants, the cardiovascular management in this group of infants remains controversial even after several years of research (Engle 2001, Dempsey and Barrington 2006).

Extremely preterm infants are at high risk of mortality and morbidity secondary to sepsis, necrotising enterocolitis, severe intracranial haemorrhage and chronic lung disease. Though neonatal care has witnessed increased survival of extremely premature infants, we have failed to see a reduction in the associated morbidities (Johnson and Marlow 2017). National UK data from 2013 showed that of the 2,686 deaths that occurred, 44% were due to respiratory and cardiovascular causes (O.N.S 2013). Cardiovascular instability early on in postnatal life along with the effects of transitional circulation and a lack of clear consensus on what constitutes low blood pressure (BP) has made this aspect of neonatal care controversial leading to wide variation in clinical practice. To appreciate the complexity of cardiovascular management for this group of infants requires a clear understanding of the fetal circulation and transitional circulation - changes occurring in the circulation at the time of birth in a term infant and comparing these changes to those occurring in an extremely premature newborn infant.

Figure 1.1: Extremely preterm infant born at 24 weeks gestation receiving intensive care in the neonatal unit of The Royal London Hospital.

Image reproduced with parental consent

Fetal circulation

Fetal circulation is unique in that it is a parallel circulation with both ventricles contributing to the systemic circulation. On the fetal side, oxygenated blood flows from the placenta through the umbilical vein into the right side of the heart via the ductus venosus. Oxygenated blood flows from the dominant right side of the heart to the left side via the foramen ovale. The oxygenated blood then flows to the upper half of the body supplying the cerebral and the coronary arteries. Less saturated blood flowing from the superior vena cava flow into the right ventricle. The majority of blood flows into the pulmonary artery, bypassing the unaerated lungs, and enters the descending aorta via the patent ductus arteriosus and supplies the lower half of the body (Figure 1.2).

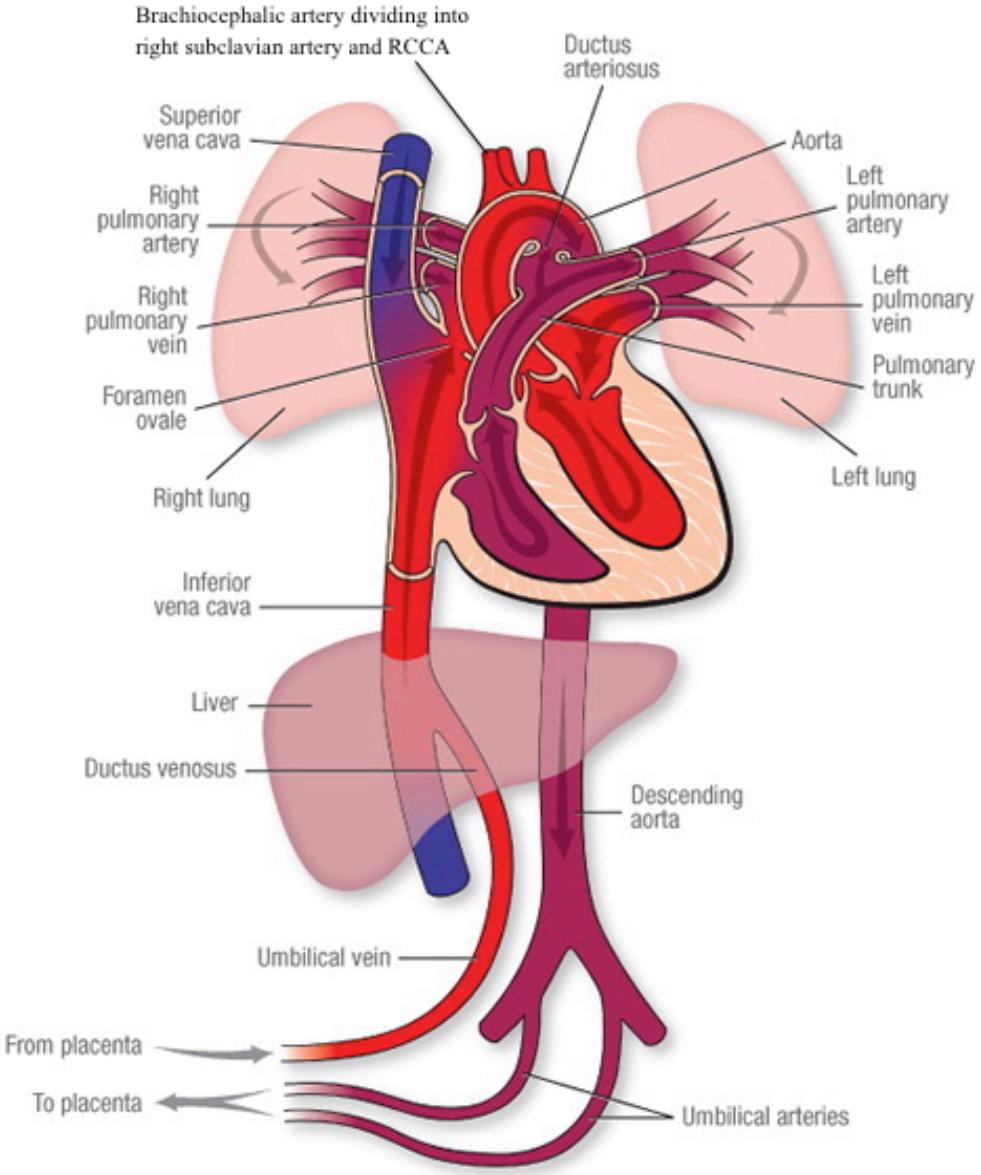


Figure 1.2: The fetal circulation
Image courtesy –American Heart Association

Transitional circulation

In the term infant, smooth transition from intrauterine to extrauterine life is initiated by occlusion of the umbilical cord and the infant crying which increases oxygenation and heart rate in response to satisfactory lung inflation. This results in increased left ventric-

ular output due to a rise in heart rate, increased left ventricular end diastolic volume due to increased pulmonary blood flow (due to reduced pulmonary vascular resistance from oxygenation), increased inotropic effect from circulating catecholamines and improved left ventricular wall compliance due to decreased right ventricular systolic and diastolic load (Anderson 1996).

In extremely preterm infants, the above mentioned physiological changes occur but the preterm myocardium makes this population more vulnerable. In contrast to term infants, the preterm myocardium has a higher water content, fewer contractile elements, greater surface to volume ratio and reliance on the L–type calcium channels which utilise extracellular calcium instead of sarcoplasmic reticulum as a messenger during cardiomyocyte contraction (Wu et al. 2016). Cord clamping results in occlusion of the umbilical vein causing a drop in right ventricular venous return and thus reducing preload by about 50%. The umbilical artery occlusion eliminates blood flow to the low resistance placental circulation causing immediate increase in the left ventricular afterload. Both the decrease in preload and increase in afterload could have a negative impact on the contractility of the already compromised preterm myocardium (Wu et al. 2016).

The dynamic nature of transitional circulation results in wide variation in BP in this group of infants (Laughon et al. 2007). This wide variation in BP makes it difficult to define a 'normal' BP range and thus manage low BP in this group of infants. Furthermore, BP is influenced by systemic flow and vascular resistance. In clinical practice, BP is routinely measured and systemic flow less so. Lastly, we know from previous work that gestational age, postnatal age and birth weight have been associated with higher BP in the more mature infant but less so in the case of the extremely premature newborn infant (Watkins

et al. 1989, Cunningham et al. 1999). The interplay of the above–mentioned reasons at varying levels makes the cardiovascular management all that more difficult in this group of infants in the first few days of postnatal life.

Blood pressure and heart rate are the most frequently measured parameters in the neonatal intensive care unit that reflects the haemodynamic status (Groves et al. 2008, Evans 2009). BP, a dependent variable, is determined by two main independent variables, flow through the blood vessel and the resistance the blood vessel offers to this flow of blood (Guyton and Hall 2006). Thus, according to the law of fluid dynamics, Δ Pressure = flow * resistance. The systemic flow or CO is the product of the heart rate and stroke volume (Figure 1.3). The stroke volume is determined by the preload (the amount of blood returning from the systemic circulation to the heart), the contractility (the pump) and the afterload (the resistance against which the heart pumps blood). Several factors previously discussed can influence the stroke volume. As preterm infants have limited ability to increase the stroke volume for reasons previously mentioned, the CO is predominantly increased by increasing the heart rate.

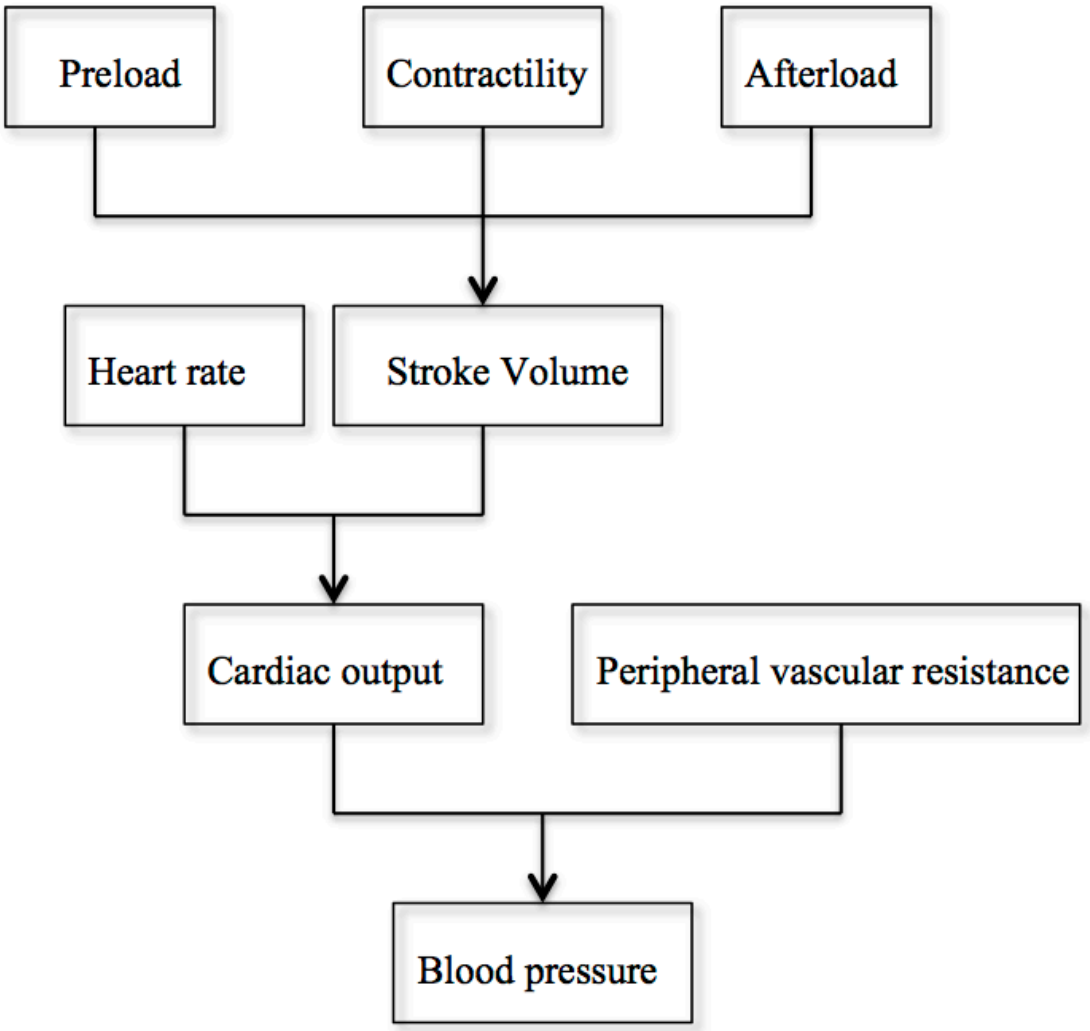


Figure 1.3: Determinants of blood pressure.

There are several differences between the term and preterm infant which affects the different parameters influencing BP (Figure 1.4). These include structural differences in the myocardium itself to other factors including sepsis, perinatal complications and inotropes. In addition to these differences, another important factor influencing the peripheral vascular resistance (and thus BP) in preterm infants is catecholamine insensitivity. We know that preterm infants have lower catecholamine levels when compared to term infants (Travers et al. 2018, Greenough et al. 1987). Glucocorticoids are known to increase beta–adrenergic receptor binding in vascular smooth muscles in animal models (Jazayeri and Meyer 1988). This typically presents clinically with refractory hypotension which resolves with systemic steroids (Fauser et al. 1993, Fernandez et al. 2005). Studies

have shown a strong positive correlation between serum cortisol and BP and a negative relationship between serum cortisol and inotropic dose (Ng et al. 2004). Catecholamine insensitivity presenting clinically as pressor–resistant hypotension has been shown to be responsive to hydrocortisone in preterm infants leading to a reduction in volume and pressor requirement (Seri et al. 2001).

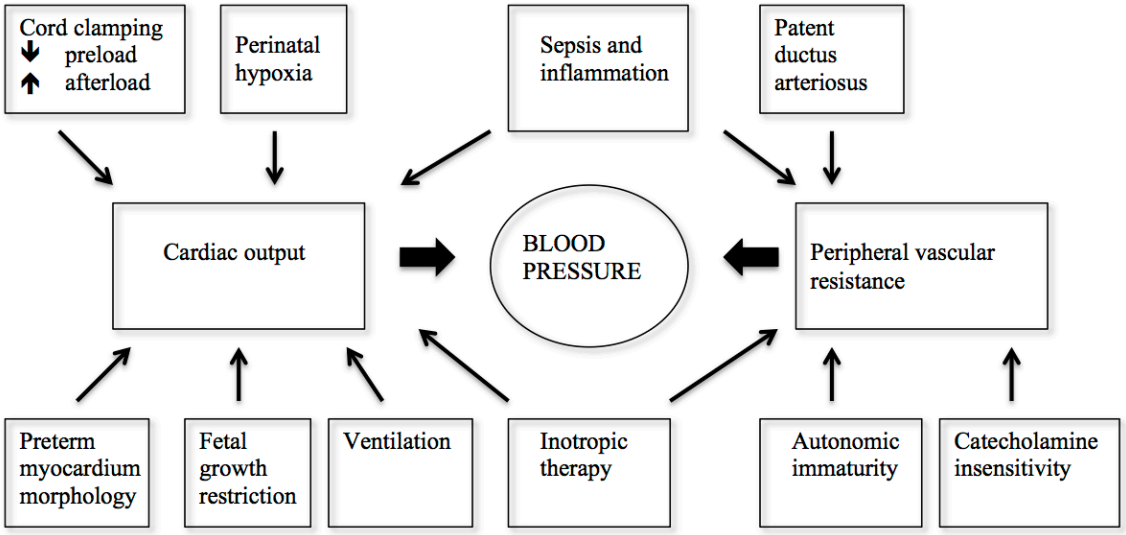


Figure 1.4: Factors affecting the cardiovascular system of the extremely preterm infant.

Peripheral vascular resistance cannot be measured but can only be derived and blood flow is not routinely measured (Engle 2001). Where blood flow or CO is not measured, it is assumed to be normal if there is evidence of metabolism or breakdown products being produced in the 'normal' range by the organ in question, for example; good urine output indicates well perfused kidneys or a normal serum lactate reflects good tissue perfusion. Clinically, BP along with surrogate markers for tissue perfusion have been used to commence and guide inotropic management in preterm infants. The markers to gauge blood flow like capillary refill time are known to have a poor correlation with BP (Osborn 2004). It is clear from the above equation that BP will be preserved if blood flow is reduced and resistance increased or during states of increased blood flow and reduced peripheral vascular resistance. Thus blood flow or CO is clinically considered to be a more

important physiological variable than BP (Kluckow and Evans 2001*b*). However, though CO measurements requires skilled personnel, it is increasingly being used in the clinical environment.

The relationship between physiological variables such as CO, cerebral blood flow and other markers of peripheral perfusion have been examined in the past using different techniques. However, these studies have been limited by small numbers (Victor, Appleton, Beirne, Marson and Weindling 2006), the use of non–invasive BP (Shah et al. 2013) and different methods to estimate cerebral blood flow (Meek et al. 1998, Victor, Appleton, Beirne, Marson and Weindling 2006, Shah et al. 2013). Victor and colleagues (Victor, Appleton, Beirne, Marson and Weindling 2006) examined 35 very low birth weight infants and concluded that cerebral fractional oxygen extraction (surrogate of cerebral perfusion) was maintained at BP above 23 mmHg. Shah and colleagues (Shah et al. 2013) examined the relationship between EEG and BP in a large number of infants but used a mixture of invasive and non–invasive BP to do this. Non–invasive BP has been known to over-estimate BP (Troy et al. 2009, Dannevig et al. 2005, Diprose et al. 1986) and may not reveal the true relationship between BP measured this way against other physiological variables. Lastly, Meek et al and Victor et al carried out work using near infra red spectroscopy (Meek et al. 1998, Victor, Appleton, Beirne, Marson and Weindling 2006) whereas Shah and colleagues used superior vena caval flow (Shah et al. 2013) to represent cerebral blood flow. Though near infra red spectroscopy is widely used and has the advantage of real time continuous information on cerebral oxygenation, it cannot be applicable to all infants as it relies on obtaining a difference in oxygen saturations to calculate cerebral oxygenation. Cerebral blood flow measurements using superior vena cava have been questioned (Ficial et al. 2017) due to the shape of the vessel and the resulting accuracy

in cerebral blood flow volume estimation. Despite several studies exploring these cardiovascular parameters, the true relationship between them remains unclear in extremely preterm infants.

Blood pressure is a commonly monitored clinical parameter to objectively assess the cardiovascular status in the preterm infant (Subhedar 2003) currently. The BP threshold for commencing inotropic support for this group of babies is very variable. Several years ago, the British Association of Perinatal Medicine (BAPM) (BAPM 1992) proposed a pragmatic approach where mean BP was maintained above the infant's gestation in weeks (for example, 26 mmHg for a baby of 26 weeks gestation). This remains the most widely practiced method for supporting BP in the United Kingdom and worldwide (Bhojani et al. 2010, Stranak et al. 2014, Dempsey and Barrington 2006) due to its pragmatic approach and the absence of strong evidence to change practice.

Permissive hypotension is another approach being practiced to support circulation. Here BP is not actively supported unless there is clinical or biochemical evidence of poor tissue perfusion (for example a poor urine output, rising serum lactate levels or worsening base excess). This form of cardiovascular support is increasingly being practiced despite the lack of any randomised controlled trial comparing this method with other types of BP support. This approach is based on work showing no relationship between BP and cerebral blood flow in sick preterm infants (Tyszczuk et al. 1998), an association between anti–hypotensive treatment and adverse outcomes (Batton et al. 2016) and a lack of association between hypotension and cranial ultrasound abnormalities/ adverse neurodevelopmental outcomes (Trounce et al. 1988, D'Souza et al. 1995, Perlman et al. 1996, Wiswell et al. 1996, Dammann et al. 2002, Limperopoulos et al. 2007, Dempsey et al. 2009, Logan,

O'Shea, Allred, Laughon, Bose, Dammann, Batton, Engelke and Leviton 2011, Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Kuban, Paneth and Leviton 2011, Alderliesten et al. 2014). Therefore, the focus has moved from BP to supporting the circulation. However, the basis of this approach is open to debate. These studies are not without drawbacks; there were retrospective studies (Perlman et al. 1996, Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Engelke and Leviton 2011, Alderliesten et al. 2014, Dempsey et al. 2009) using non–randomised comparisons (Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Kuban, Paneth and Leviton 2011, Alderliesten et al. 2014). The lack of data on BP method used (Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Kuban, Paneth and Leviton 2011, Alderliesten et al. 2014) and including non–invasive BP (Dempsey et al. 2009, Trounce et al. 1988, Perlman et al. 1996) which is known to over–estimate BP may influence final results. Tyszczuk and colleagues (Tyszczuk et al. 1998) examined a short period of cerebral circulation which may not reveal the true relationship between BP and cerebral blood flow. The relationship between low BP and intraventricular haemorrhage is also conflicting with several studies failing to show an association (Miall-Allen et al. 1987, Watkins et al. 1989, Bada et al. 1990, Low et al. 1992, 1993, O'Shea et al. 1998). Furthermore, a lack of association between treatment and adverse outcomes has been reported (Batton et al. 2009). Practically, CO measurements requires the availability of skilled clinicians to perform and monitor response to treatment. The above arguments demonstrates that adopting this approach to support circulation is not based on sound scientific evidence and is not without its challenges.

Another approach to BP management is actively maintaining mean arterial BP above 30 mmHg. This approach is based on the results of prospective studies using invasive

mean arterial BP which demonstrated reduced cerebral blood flow (Munro et al. 2004), increased white matter damage (Miall-Allen et al. 1987) and lower serum creatinine levels (Thayyil et al. 2008) when mean arterial BP was less than 30 mmHg. Furthermore a recent large trial examining the short term outcomes of hypotension in nearly 5,000 babies found hypotension to be associated with increased mortality and morbidity (Faust et al. 2015). Though this method of supporting BP is only followed by a small number of neonatal units (Dempsey and Barrington 2006), it is based on prospective studies using invasive BP data. Some reasons for only a minority of neonatal unit to continue this method of BP management (despite evidence from several years ago) include; lesser cranial ultrasound abnormalities and therefore improved neurodevelopmental outcomes in these neonatal units, relatively lesser mortality and morbidity compared to other units and lastly, the absence of good scientific data (randomised controlled trials) to trigger change in practice.

Our tertiary level neonatal intensive care unit at The Royal London Hospital actively supports BP. We maintain the mean arterial BP above 30 mmHg in extremely preterm infants. This threshold was chosen as a result of studies showing reduced cranial ultrasound abnormalities and improved end organ perfusion above this threshold as discussed in the above–mentioned studies. In addition, clinicians reported lower rates of cranial ultrasound abnormalities (personal communication) and lower serum creatinine levels (Thayyil et al. 2008) in this cohort of infants when compared to other infants.

In order to tease out if actively supporting BP per se was associated with increased mortality, we compared our east London cohort of infants with the Montreal cohort of infants where permissive hypotension (Dempsey et al. 2009) was being followed over a similar

time period. The results of these are shown in study protocol (Appendix, 7.1, Table 1). When compared to Montreal, the overall mortality was not significantly different in the east London cohort (28% vs 21%, p=0.21). The number of infants receiving inotropes were significantly higher in the east London cohort when compared to Montreal (69% vs 15%, p<0.001). When comparing clinical outcomes among infants receiving inotropes, mortality was found to be significantly lower in the east London cohort (25% vs 72%, p<0.001). However, it would be difficult to use non–randomised comparions between units to determine which approach to BP support is superior. The most ideal way to answer these questions is to carry out a randomised controlled trial.

The criteria for supporting low BP varies widely between various neonatal units with lack of good scientific data to suggest the best method for supporting BP. There has been lot of work comparing the different drugs used to support BP (Osborn et al. 2002, Greenough and Emery 1993, Rozé et al. 1993, Subhedar and Shaw 2000), but there is a paucity of randomised studies comparing BP intervention levels. This has been identified as an area of priority for future neonatal research (Short et al. 2006). There also remains considerable debate as to the best way to support a preterm circulation (Evans 2009) and what parameters, both clinical and echocardiographic, would accurately reflect the infant's cardiovascular status. There is an increasing trend among clinicians to use BP along with systemic blood flow measurement to make an informed decision regarding what further haemodynamic management would be most appropriate. These questions will need to be answered using a well-designed randomised controlled trial that investigates BP intervention levels for extremely preterm infants and to examine if adverse neurodevelopmental outcome is the result of low BP, effects of poor organ perfusion or treatment used to support low BP.

To investigate if any such randomised trials in this area have been carried out in the past, a systematic search of the literature was performed earlier and repeated on the 20th April 2017 using the Healthcare Database Advanced Search database. The following terms (infant OR infant, premature OR infant low birth weight OR extreme prematurity OR preterm infant) AND (blood pressure OR arterial pressure OR BP OR mean blood pressure) AND (randomised controlled trial OR randomised controlled trial OR RCT) were entered and searched using Medline, EMBASE and Open Access Theses and Dissertations with no restrictions. This search yielded 33 results in total. After initial screening and removing duplicates we were left with two relevant articles (Figure 1.5).

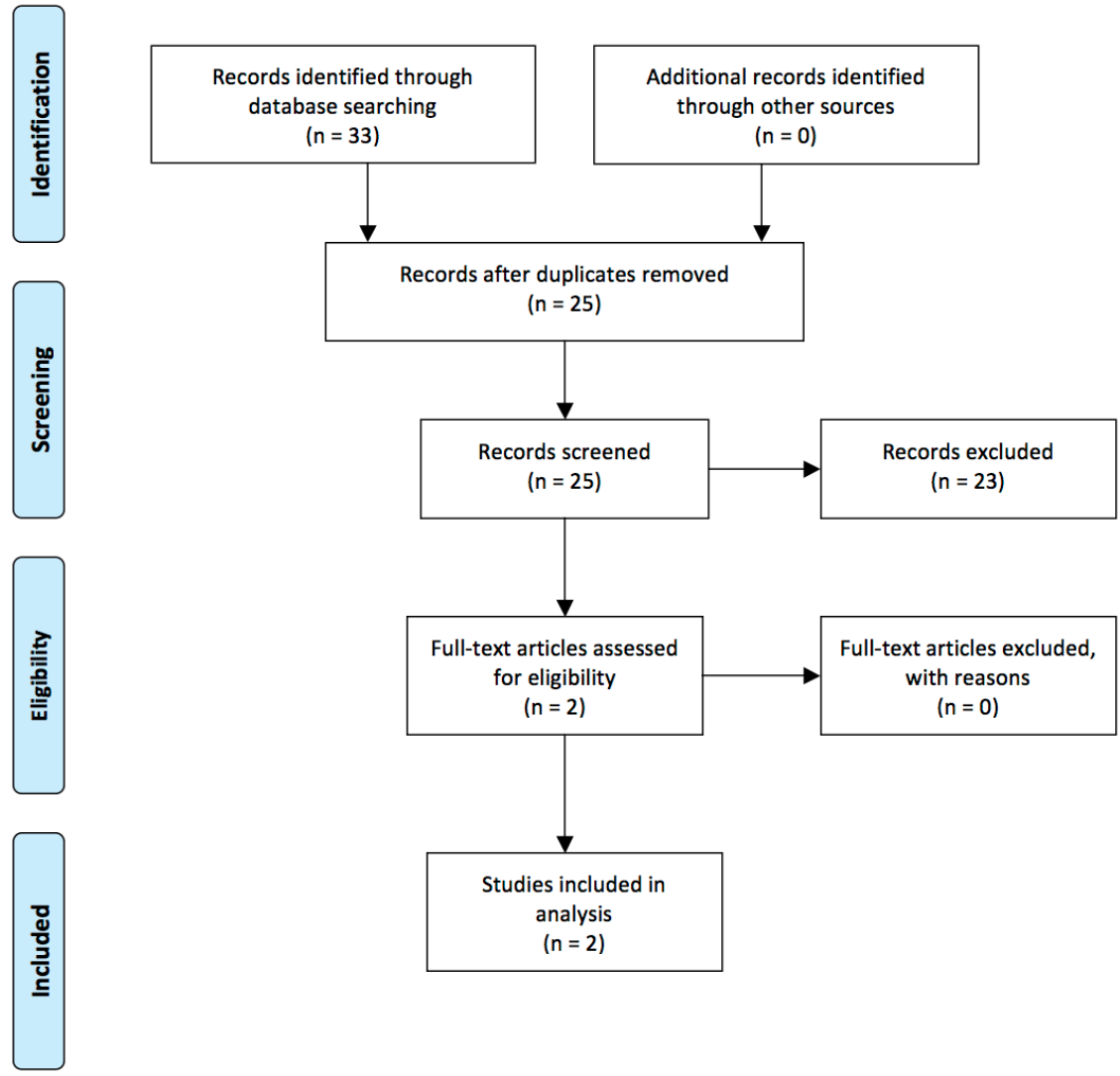


Figure 1.5: PRISMA flow diagram showing the initial results from systematic search. *(From Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses.)*

Among the two studies listed in the search, one study reported their finding whilst the other is currently recruiting. Batton and colleagues (Batton et al. 2012) carried out a randomised controlled trial for BP management. They reported their findings on the premature termination of the trial due to poor recruitment (10 out of the planned 60 infants (17%) recruited).

To date, to the best of our knowledge, there are no trials comparing BP levels which were

higher or lower than the most commonly used intervention level in the United Kingdom nor were there any completed randomised controlled trials for BP management to report their results.

1.2 Hypotheses

i. Alterations in blood pressure and cardiac output has an effect on cerebral blood flow.

ii. These cardiovascular measures are directly related to EEG measures of continuity.

iii. Different approaches to blood pressure intervention will result in different usage rates of inotropic agents and levels of achieved blood pressure, in extremely premature newborn infants [less than or equal to 28^{+6} weeks gestation].

1.3 Aims

1) To understand the relationship between the cerebral blood flow, cardiac output and amplitude integrated electroencephalographic activity in extremely preterm infants.

2) To perform validity and reliability work on the physiological measurements performed in the study.

3) To compare different levels of BP at which staff caring for extremely premature newborn babies should intervene to start treatment. This is a pilot study, which will determine whether Active, Moderate or Permissive BP support regimens results in different achieved BP levels and different rates of inotrope usage. The data collected in this study will determine whether a larger multicentre study would be feasible and justified. Secondary

outcome measures will be collected to inform power calculations for important clinical outcomes in a larger study. Three approaches to intervention will be compared in 60 babies' born between 23^{+0} to 28^{+6} weeks gestation:

i. Active: Mean arterial BP (MABP) was supported if it fell below 30 mmHg for more than 15 consecutive minutes

ii. Moderate: MABP was supported if it fell below the infants gestational age in mmHg for more than 15 consecutive minutes

iii. Permissive: MABP was supported if it fell below 19 mmHg for more than 15 consecutive minutes, or if there was clinical evidence of impaired tissue perfusion (such as reduced urine output <1 ml/kg/hour, poor skin perfusion, worsening base excess or rising serum lactate).

1.4 Pilot study

A pilot study is a 'small study for helping to design a confirmatory study' (Arain et al. 2010). Thabane and colleagues (Thabane et al. 2010) provide a useful summary for the reasons for performing a pilot study. This has been grouped into several broad classifications;

i. Process: This assess the feasibility of steps required to carry out the main study. Examples include recruitment rates, retention rates.

ii. Resources: This deals with assessing time and budget issues. The main aim of this work is to collect useful data on the length of time required to fill data, for example survey forms etc.

iii. Management: This covers personnel and data management at participating centres.

iv. Scientific: This deals with assessment of treatment safety, estimation of treatment effect and its variance.

Pilot or feasibility studies give useful information to researchers who are doing similar work and provide information for sample size calculation. Pilot studies have a separate consolidated standards for reporting trials as well (Eldridge et al. 2016).

1.5 Measurement of physiological parameters

The main aspect of cardiovascular support this randomised controlled trial will be focussing on is BP. In addition, this study will examine other physiological markers of perfusion such as left ventricular output (cardiac output), right common carotid artery blood flow (as a surrogate of cerebral blood flow), superior mesenteric artery blood flow and markers of end organ function such as amplitude integrated electroencephalography. Unlike other cerebral Doppler studies which have reported on blood velocities, this study will report cerebral blood flow volumes (using blood flow velocity and blood vessel diameter) which would give more meaningful results in this group of extremely preterm infants.

In the sections to follow, I will discuss the various methods available for measuring these physiological parameters and give reasons –for and against and why a particular method was chosen in this study.

1.6 Methods for measurement of cardiac output

There are several invasive and non–invasive methods for measuring cardiac output (CO) described throughout literature. This section discusses these methods which have been

summarised in Table 1.1.

1.6.1 Fick's Cardiac output measurement

Fick's direct oxygen method

Adolf Fick first described this method of estimating CO in 1870. This method works on the principle of estimating the amount of oxygen (as well as carbon dioxide) used by the body. Once an identified amount of oxygen is delivered to the body, cardiac output is obtained by calculating the difference in oxygen content between arterial and venous blood. The equation below summarises how cardiac output is calculated:

$$CardiacOutput(CO) = \frac{VO2}{(CaO2 - CvO2)}$$

VO2 –the oxygen uptake, CaO2 –the oxygen content in arterial blood (ml O2 per litre) and CvO2 –the oxygen content in the venous blood (ml O2 per litre) (Geerts et al. 2011).

This method is considered to be the gold standard against which most other methods are compared. The amount of oxygen delivered can be measured through the mechanical ventilator; an invasive pulmonary artery Swan-Ganz catheter is required to measure the mixed venous blood. This method, though invasive, is relatively easy and has minimal operator dependency. However, it is not without limitations. The entire process can be quite laborious and time consuming to be carried out at the cotside. There are quite a few variables, which could increase the chance of errors. This method requires a stable supply of oxygen. Fluctuations in oxygen supply and an oxygen requirement of >60% is associated with errors. Lastly this method relies heavily on the lungs to effectively utilize oxygen (Axler et al. 1996). The ability to use oxygen effectively by the lungs and errors as-

sociated with a high FiO2 could be a limiting factor for the use of this method in my study.

As my subjects are extremely preterm infants who have a 90% incidence of respiratory distress syndrome (Horbar et al. 2010) predisposing to hypoxia and hypercapnoea, fluctuating oxygen requirements and unreliable lung efficiency as far as gas exchange is considered renders this method unreliable for estimating CO in this study.

1.6.2 Indicator dilution techniques

Fegler (Fegler 1954) first described this method using dogs. The principle involves injecting an indicator into the blood and measuring its levels over a period of time. The first step to doing this is to calculate mass balance using the formula:

$$mi=\int q(t)\times c(t)dt$$

mi–amount of indicator injected

q(t)–instantaneous blood flow

c(t)–concentration as a function of time

Cardiac output is calculated using the Stewart - Hamilton equation that is as follows;

$$CO=\frac{mi}{\int c(t)dt}$$

CO –cardiac output

mi –amount of indicator injected

∫c(t) dt –represents area under the indicator dilution curve

There are four different dilution techniques commercially available. These include pulmonary artery catheter bolus and continuous thermodilution, transpulmonary thermodilution and lithium bolus dilution methods (Geerts et al. 2011). These methods assume

complete mixing of blood with the indicator.

Pulmonary thermodilution

This technique involves the bolus administration of a cold fluid via a Swan–Ganz catheter very close to the right atrium and the resulting dilution curve is detected in the pulmonary artery via a thermistor. The CO is then measured using the Stewart—Hamilton equation stated earlier.

This clinical method of estimation of CO is generally what other methods are compared with. For improving accuracy an average of 3 to 4 thermodilution measurements are taken. The advantages of this method include repeatability which is fast and easy to perform. The disadvantages include complications associated with an invasive procedure like risks of perforation, arrhythmias. This method is unreliable in the presence of tricuspid regurgitation and right to left shunt, which will cause error in the estimation of the CO (Axler et al. 1996). In the first few days after birth, transitional circulation characterised by tricuspid regurgitation and presence of right to left shunts makes this method unreliable to use for the purposes of this study.

Transpulmonary lithium thermodilution

This technique utilizes the same principle as bolus thermodilution but uses lithium chloride. Several measurements are required for accurately estimating the CO which entails multiple blood tests. It is not licensed for use in any patient weighing <40 kg (Geerts et al. 2011).

This method has been in use in paediatric population for some time. One case series involved twenty–four children with the smallest infant weighing 2.6 kg (Linton et al. 2000). No adverse events were reported, but the minimum blood volume for each sample was

3 millilitres. The recommendation, for each estimation, is to average results from 3 to 4 samples to improve accuracy. This entails drawing at least 12 millilitres of blood per measurement. Extremely preterm infants have a total blood volume of about 70 ml/kg (Aladangady et al. 2004). Given the mean weight of infants in my study is approximately 800 grams; this would amount to 20% of the total blood volume for a single estimation of CO, which is a significant amount of blood making this method impractical for this study.

1.6.3 Pulse contour cardiac output

This method calculates CO indirectly. It measures the beat-to-beat stroke volume using a mathematical model, the Windkessel model first described by Otto Frank (Frank 1899). This is achieved by inserting a arterial catheter ideally in the aorta to monitor the arterial waveforms. The resulting area under the waveform is what contributes to the calculation of the CO. The signal can be dampened if a peripheral artery is used.

This method is scantly validated when compared to other methods like thermodilution for which there is a clear understanding of how the CO is measured. The lack of understanding of how this method calculates the CO based on a mathematical model is open to debate. Therefore, this is not ideal for measurement of CO.

1.6.4 Electrical bioimpedance and bioreactance

Bioimpedance is a non–invasive method of estimating CO that has been used for several years. A pair of electrodes placed on the chest delivers a low amplitude current. Another pair of electrode placed in between this pair of electrode picks up voltage, which is thought to be due to bioimpedance from the cardiac blood volume. The CO is derived after computation using the difference in voltage.

Bioimpedance is scantily validated and several factors could influence the results. One of the important factors is movement artefact, environmental noise and the presence of shunts and cardiac abnormalities. The presence of movement artefact is significant because preterm infants have lot of involuntary movements on minimal handling which could influence the results. Environmental noise could contribute significantly to errors in estimation of the CO. The above mentioned reasons make this a less favourable method for use in this study.

Bioreactance examines the change in the amplitude and frequency of voltage as it traverses through the thoracic cage (Mateu Campos et al. 2012). The benefit of this being that it reduces the interference from environmental noise and movement artefact. However shunting in the heart leads to errors and makes this unreliable for use in extremely preterm infants in the first few days of life. Moreover, there is very little validation of this method for use in this group of patients.

1.6.5 Doppler ultrasound

Doppler Ultrasound –History

Christian Johann Doppler, an Austrian mathematician and physicist first described the Doppler effect in 1842 (Roguin 2002). The Doppler effect is the apparent difference between the frequency at which waves (sound or light) leave a source and that at which they reach an observer, caused by relative motion of the observer and the sound wave (Roguin 2002, McNay and Fleming 1999). There has been widespread use of the Doppler effect in medicine, radar technologies and weather forecasting. The first use of ultrasound in medicine was by Karl Dussick, an Austrian neurologist, in 1951(Edler and Lindstrom 2004).

Doppler ultrasound –physics and instrumentation

A) Sound waves:

Sound waves are mechanical impulses of kinetic energy (vibrations) that can be described in terms of their frequency in cycles per second or Hertz (Hz), their wavelength in terms of millimetres (mm) and amplitude in terms of decibels (db). The human ear can hear sound waves in the range of 20 Hz to 20,000 Hz (HPA 2010). Sound waves with a frequency lower than 20 Hz are known as infrasonic waves whereas sound waves with a frequency greater than 20 KHz are called ultrasound waves. Sound waves require a medium to travel through. The velocity of sound in air is 330 metres per second as compared to human tissue where it is 1540 metres per second (Curry et al. 1990). The waves propagate through a medium by alternating between compression and rarefaction (Otto 2000). Sound waves with a long wavelength have a higher penetration but at the expense of resolution. Short wavelengths offer the best resolution possible.

B) Doppler Equation:

The Doppler effect in the tissues can be expressed as an equation, which is as follows:

$$V = \frac{(Fd \times c)}{(2 \times Fo \times cos\theta)}$$

V –velocity of blood

Fd –change in frequency (Doppler shift)

c –velocity of ultrasound in blood

Fo –transmitted frequency

cos θ –angle between the direction of ultrasound beam and blood flow

The angle of insonation (cos θ) is the angle between the direction of blood flow and the ultrasound beam. This has an important practical application. As cos 0° is 1 and cos 90° is 0, obtaining a low angle of insonation is important to obtain the most accurate results. Ideally the angle of insonation should be as small as practically possible (but not $\geq$60°) using an angle corrected sampling gate.

Transthoracic Doppler ultrasound

Transthoracic Doppler ultrasound is a non-invasive tool that is freely available in most neonatal units. Its portable nature means that measurements can be carried out at the bedside with ease. It is increasingly used as an adjunct to clinical assessment in intensive care (Mertens et al. 2011) since it provides instantaneous feedback and helps clinicians to fine tune cardiovascular management (Burdjalov et al. 2002). It is generally well tolerated with no significant changes in the heart rate, BP or oxygen saturations (Groves et al. 2005, Noori and Seri 2014). Though this method is widely available, it is subject to intra and inter observer variability and practitioners require the necessary skills before it can be used accurately. Other advantages include being non-invasive, reproducible and widely available in most neonatal units. I have therefore decided to use this method of investigation as my preferred choice for cardiovascular assessments in this study.

Transoesophageal Doppler ultrasound

Transoesophageal method of assessment is more commonly used in the adult setting. It has its advantages in obtaining better images especially of the root of the aorta. The use of this in the preterm newborn infants is difficult due to the presence of nasogastric tube and endotracheal tube being in situ. This is relatively more invasive and hence more likely to disturb the infant than the transthoracic method and technically challenging in this group of infants.

1.6.6 Magnetic resonance imaging

Magnetic resonance imaging has been validated for use in both adults and children (Powell et al. 2000). There has been further development with this method and recently has been applied to preterm and term infants (Groves et al. 2011). MRI has since been used in preterm infant with no adverse events. Phase contrast MRI has been used to measure CO. This has been shown to correlate well with echocardiography as well (Ficial et al. 2013).

Though this method has good correlation, it is not without challenges. It is expensive and impractical to use for this study for several reasons; firstly, the scanner should be readily available ideally within the neonatal unit. Secondly, it may not reflect acute changes due to the time lag in obtaining the scan. Thirdly, it involves moving the baby to the scanner, the need for monitoring and respiratory support equipment, which is MRI compatible. This method entails a lot of organisation and handling of the baby which in the early hours of life would cause a lot of instability at a period when these babies need stability with minimal handling. MRI is also expensive to perform. Hence, despite of good correlation with echocardiography, this method was not chosen.

Method	Advantages	Disadvantages
Fick's method	Considered gold standard with minimal operator dependency	Invasive procedure, laborious, stable supply of oxygen required and lungs should effectively utilise oxygen
Indicator dilution technique	Good repeatability and easy to perform	Invasive technique, multiple measurements required, unsuitable in the presence of tricuspid regurgitation
Pulse contour technique	Measures beat to beat stroke volume	Invasive technique, dampening of signal if peripheral artery used
Electrical bioimpedance	Non invasive technique	Scantily validated, results influenced by motion artefact
Doppler ultrasound	Non invasive, widely available, easy to perform, compatible and can be performed several times at cot side	Operator dependent, learning curve, physics of sound waves
Magnetic resonance imaging	No adverse events, validated for use in preterm infants, correlates well with Doppler ultrasound	Scanner required within the neonatal unit, requires compatible respiratory and monitoring equipment, excessive handling of infant, results may lag behind circulatory changes, repeated measurements cumbersome, more personnel required and is expensive.

Table 1.1: Summary of the comparison of advantages and disadvantages between the various methods to measure cardiac output.

1.7 Comparison between measurement of cardiac output using Doppler ultrasound with other methods

1.7.1 Doppler ultrasound versus thermodilution

Mellander and colleagues (Mellander et al. 1987) studied 10 children aged between 6 weeks to 13 years who did not have intracardiac shunts. With each of the six consecutive thermodilution injections, simultaneous CO measurements using Doppler ultrasound were performed. A good correlation was found, r = 0.97 between thermodilution and CO measured by Doppler ultrasound.

1.7.2 Doppler ultrasound versus cardiac catheterisation

Alverson and associates (Alverson et al. 1984) studied 8 preterm infants and 14 term infants. CO measurements using Doppler ultrasound were performed and normative values were obtained for this group of infants. The values of CO using Doppler ultrasound in this study were similar to other studies that used cardiac catheterisation and thermodilution to measure CO in healthy newborn infants.

1.7.3 Doppler ultrasound versus magnetic resonance imaging

Echocardiography is the most commonly utilised method for estimating CO in clinical practice. Where there is the necessary infrastructure to perform MRI to evaluate the CO, this would be ideal due to the superior repeatability seen in the newborn infants (Groves 2011). One study comparing CO measured by phase contrast MRI and echocardiography (Ficial et al. 2013) in 49 infants with a median gestation and birth weight of 34 weeks and 1880 grams respectively showed a strong correlation ($R^2 = 0.83$) between the two methods. Mean bias between phase contrast MRI and echocardiography using Bland-Altman was -9.6 ml/kg/min with the LoA being between of -79.2 ml/kg/min to +60 ml/kg/min.

1.8 Methods for measurement of cerebral blood flow

There are several methods of measuring cerebral blood flow in the newborn infant described throughout the medical literature (Pryds and Edwards 1996). In this section, I have discussed the various available methods, provide reasons for the chosen method and have summarised these in table 1.2.

1.8.1 Nitrous Oxide Technique

Kety and Schmidt first described this method in the 1940's using the Fick principle. This principle states that the uptake and clearance of an inert diffusible gas (inhaled nitrous

oxide) is proportional to the blood flow. One of the major drawbacks for this method was the requirement of large volumes of blood. The other limitation of this method was that the equilibrium of nitrous oxide between the brain tissue and circulation occurred very slowly (Vutskits 2014). This lag in results meant that this method did not accurately reflect acute changes in BP and cerebral electrical activity. These reasons made this method unreliable for estimation of cerebral blood flow for this study.

1.8.2 ^{133}Xe Clearance Technique

The principle of this method is the measurement of the rate of clearance of an inert radioactive tracer. The tracer is either inhaled as a gas or injected into blood so that it enters the cerebral circulation. Being an inert agent, it is not metabolised and is removed from blood by diffusing back into the cerebral circulation. The rate of clearance will be proportional to the cerebral blood flow. The major drawback of this technique is the use of radioactive substance. One measurement involves up to 0.2 mGy (20 mrad), which is equivalent to two chest x-rays. Secondly, the measurements do take time and are not reflective of instantaneous changes in the cerebral circulation. Though this method has been used in the neonatal unit previously, the use of radiation is a major disadvantage thus making this method less preferable for repeated measurements.

1.8.3 Positron Emission Tomography

This method involves the use of ionising radiation to measure the regional cerebral blood flow, oxygen and glucose uptake of the brain using positron emission tomography. Each of these estimations could involve up to 0.57 mGy (57 mrad). This test also requires a considerable amount of blood to be taken for measurement of each cerebral blood flow. The exposure to relatively large amounts of radiation and the blood test volumes makes this method inappropriate to use in extremely preterm newborn infants.

1.8.4 Xenon Computed Tomography

This method uses non-radioactive Xenon as a marker. Xenon is inhaled and blood concentration is estimated using computed tomography. The doses of Xenon used for this investigation could potentially have an anaesthetic effect or cause increased intracranial pressure. This investigation cannot be done at the bedside. It has also not been studied well in newborn infants and therefore is not considered for estimation of cerebral blood flow in this study.

1.8.5 Near Infrared spectroscopy

Near Infrared spectroscopy (NIRS) is a method that has been well established and is commonly used in the neonatal unit to interrogate the cerebral blood flow. NIRS works on the principle of the relative transparency of intact biological tissue to light in the infrared spectrum. It measures regional cerebral oxygen saturation (rcSO2) from which cerebral fractional tissue oxygen extraction (cFTOE) is calculated. cFTOE represents a balance of oxygen supply on the one hand and oxygen consumption on the other. On the assumption that the oxygen consumption of a preterm brain is relatively constant, fluctuations of cFTOE would indicate fluctuations of oxygen supply, and hence of cerebral blood flow (Pellicer et al. 2002, Kooi et al. 2013).

Though this widely used method allows continuous measurement of cerebral blood flow, it is not without limitations. The results could be influenced by the presence of motion artefact which (Quaresima et al. 2012) is common in these infants during physical examination, procedures and handling of the infant during cares. It relies on producing a difference in oxygen saturation, therefore cannot be reliably used if the infant is ventilated in air or 100% oxygen. Some of the other limitations include attenuation of the infrared light by extra-cerebral tissue and dark colour of hair, stable contact between the optical fi-

bre and skin is crucial, placement of multiple optical fibre effectively onto the scalp which could be time consuming and technically challenging in an extremely preterm infant who is ventilated (all ventilated infants in our neonatal unit wear a hat to which the endotracheal tube is secured). Lastly, it requires personnel with the necessary skills to setup, capture, analyse artefact free signal and to interpret the results. This method is more suitable in neonatal units with clinicians who have had prior experience of using NIRS.

1.8.6 Doppler Ultrasonography

Doppler ultrasound works on the principle of a shift in frequency when ultrasound is reflected from moving blood cells and this shift is proportional to the blood velocity (Pryds and Edwards 1996). Doppler ultrasound has been previously used to investigate cerebral blood flow velocity (Rennie 1989) and more recently cerebral blood flow volumes (Sinha et al. 2006) have been measured, which produced comparable results to the ^{133}Xe clearance method for cerebral blood flow estimation. The main advantage of Doppler ultrasound being that it reflects acute changes in circulation and is a reproducible method (Sinha et al. 2006) that can be easily done at the bedside which involves minimal handling of the baby with no exposure to radiation. An ultrasound machine is widely available in most neonatal units and measurement can be done repeatedly to reassess during extremes of BP and monitor response to treatment. These are the main reasons why I have chosen Doppler ultrasonography as my main method of estimating cerebral blood flow over other methods.

Doppler ultrasonography will be used for the measurement of both the right common carotid artery blood flow volume estimation and also the measurement of CO using techniques that are well established in the literature. However, Doppler ultrasonography is not without its weaknesses. In the following paragraphs, I have explored its weaknesses, limitations and challenges and how I plan to overcome these from a practical point of view

in this study.

Method	Principle	Advantages	Disadvantages
Nitrous oxide technique	Uptake and clearance of inert tracer	Uses inert diffusable gas and based on the Fick's principle	Invasive procedure, laborious, large volumes of blood sampling required, lag phase as equilibrium between brain tissue and circulation occurres slowly
^{133}Xenon clearance technique	Uptake and clearance of inert radioactive tracer	Inert substance therefore clearance will reflect cerebral blood flow	Invasive technique, exposure to radiation
Positron emission tomography	Measuring regional cerebral blood flow using ionising radiation	Easy to perform	Invasive technique, multiple measurements required which involves exposure to radiation
Xenon computed tomography	Non–radioactive tracer uptake	Well studied in newborn infants	Exposure to radiation, effect on intracranial pressure
Near infrared spectroscopy	Measures regional cerebral oxygen saturation from which CFOE is extracted	Well established and commonly used in neonatal intensive care, assumes oxygen consumption of preterm brain is constant	Prone to motion artefact, cannot be used if infant is requiring 100% oxygen, measurements likely to be affected if poor contact with scalp, technical challenges
Doppler ultrasound	Shift in ultrasound frequency from moving blood cells is proportional to blood velocity	Non invasive, widely available, easy to perform, compatible and can be performed on multiple times at cot side	Operator dependent, learning curve, physics of sound waves

Table 1.2: Summary table comparing the various methods to measure cerebral blood flow.

Measurement of cerebral blood flow volumes using Doppler ultrasound of the right common carotid artery is not widely used as compared to the other commonly practiced methods to estimate cerebral blood flow in this group of infants. A few reasons could account for this. Firstly, this measurement is most appropriately performed using the hockey–stick transducer rather than transducers used for echocardiography and cranial ultrasound scans. Secondly, using Doppler ultrasound is operator dependent and involves a steep learning curve which can be overcome using Doppler flow phantoms. Thirdly, meticulous attention to detail is required during measurement of vessel diameter in this group of infants as they are of the order of 2 mm. Lastly, lack of appropriately trained personnel in performing Doppler ultrasound makes this method less widely used than the other methods which are non–operator dependent. Doppler ultrasound, though easily performed, is not without its challenges and I have detailed these in the following pages and how to reduce the errors associated with measurement.

1.9 Technical challenges with the use of Doppler ultrasonography

1.9.1 Operator dependent

Doppler ultrasonography is increasingly being used in neonatology for various purposes (Guan et al. 2016, Kim et al. 2010, Kuschel et al. 2008, Arai and Yamashita 2005, Hirsimaki et al. 1991, Sevely et al. 1991). It is operator dependent and involves a learning curve before an operator can confidently use it. After undertaking formal training, I have performed cranial ultrasound for the last several years and have in the vast majority of scans used Doppler ultrasound to interrogate small calibre blood vessels such as the anterior cerebral artery. In addition to this, I have had formal training in performing echocardiography and also have performed several echocardiograms under direct supervision prior to the start of this study. I have also had direct supervision of performing cerebral blood flow measurements using the right common carotid artery. This involved measuring the velocity time integral and blood vessel diameters, which is a frequent source of potential error. As a part of this study, I have carried out validity and reliability studies using a Doppler flow phantom and compared my findings with that of other operators who have several years of experience.

1.9.2 Measurement errors

The diameter of the blood vessels measured are of the order of 2 mm, therefore potential areas for error could be underestimating or overestimating the diameter of the vessel. This could produce significant error due to the squaring effect when the area of the vessel is calculated. To reduce the chance of this occurring, I repeated the measurements 5 times and computed the mean of these 5 readings. Other potential areas of error could be movement artefact, for example if baby has hiccups, movement artefacts of limbs, breathing move-

ments etc. This is overcome by sampling a long enough period of waveform ensuring it is a good waveform with good sound signal. Adjusting the wall filter to a minimum level will also help to capture low velocity flow and reduce vessel wall motion artefacts (Bulwer 2011). The velocity time integral is traced automatically by the ultrasound machine for carotid blood flow but manually traced when the left ventricular output is measured. One potential source of error is tracing beyond the border of the wave envelope or tracing well within the border of the waveform. This could lead to overestimation or underestimation respectively of the velocity time integral and subsequently miscalculate the CO. This is minimised by taking the mean of five waveforms. As for the carotid blood flow waveform, when using the linear probe, the machine automatically measures the time averaged mean velocity. Doppler assessment of blood flow velocity is most accurate when the angle of insonation (angle formed between the transducer ultrasound beams and the blood vessel) is ideally less than 60 degrees. The larger this beam-vessel angle or Doppler angle, the higher would be the underestimation of the true blood flow velocity (Bulwer 2011). Selecting a part of the vessel where the least Doppler angle could be obtained will reduce this potential error. Tilting the head gently to the left side and selecting an appropriate area of the blood vessel will aid in reducing the Doppler angle. Using a wedge or a thick application of ultrasound gel will also help to modify the angle of the probe on the surface of the skin, thereby achieving the lowest possible angle of insonation.

1.9.3 Nyquist Limit

As this study involves investigating the transitional circulation of the extremely preterm infant, it is highly likely that extremes of blood flow velocities will be encountered. Pulse wave Doppler will be used to study blood flow velocities less than 2 meters per second and continuous wave Doppler will be used to study blood flow velocities greater than 2 meters per second. Pulse wave Doppler works by the ultrasound probe transmitting a se-

ries of pulses of sound energy. The phase of returning signals are compared with the phase of the emitted one by the transducer. The phase of the signal sent will be different from the phase of the signal received. A change in this phase translates to a shift in frequency. The number of these pulses discharged per second is known as the pulse repetition frequency (Torp-Pedersen and Terslev 2008). The pulse repetition frequency (PRF) being the Doppler sampling frequency of the transducer is expressed in terms of Hertz. The pulse repetition frequency is short if the area of interest is close to the ultrasound probe and the pulse repetition frequency is long if the area of interest is further away from the ultrasound probe. Pulse wave Doppler is useful in interrogating specific areas where the sampling gate is placed. There are two main limitations of the pulse wave Doppler technique (Lawrence 2007). The first limitation is the velocity limitation or the Nyquist limit. The Nyquist limit is half the sampling frequency or PRF/2. If the blood velocity is higher than the set Nyquist limit, the ultrasound machine will misinterpret the velocity resulting in aliasing. The second main limitation of the pulse wave Doppler is its inability to accurately estimate the direction and velocity of blood at excessive distances.

The above problems could be frequently encountered during my estimations of blood flow velocity especially through the patent ductus arteriosus. This being a closing vessel could generate high velocities of blood flow. The way to overcome this issue would be to set the scale at a higher velocity such that the pulse waveform could be seen in its entirety in one screen. The second option to tackle this is using the continuous wave Doppler signal. This would capture blood flow velocity in excess of 2 meters per second, which are the typical velocities for some of the velocity through the patent ductus arteriosus for some of the babies.

1.9.4 Aliasing

The use of colour Doppler gives valuable information on the direction of flow of blood through a vessel. The advent of ultrafast microprocessors and enhanced computer technology make this mode of interrogating a blood vessel highly useful tool. To use colour flow or colour mapping, the user selects the area of interest and then switches on colour Doppler function. This will highlight the vessels with blood flow through it in colour. Once the colour Doppler images are obtained, it is ideal to reduce the frame size to interrogate the area of interest as numerous computational changes take place. This could potentially slow the frame rate and thus less likely to reflect on going changes more accurately.

Once the colour Doppler mode is switched on, a colour legend will appear on the screen. The velocity of blood is represented by the varying intensities of red and blue. Conventionally, red flow represents flow towards the probe and blue represents flow away from the probe. The ultrasonographer has the option to reverse this if required. Aliasing which represents turbulent flow is one the main problem that the ultrasonographer could encounter whilst using the colour mapping (Lawrence 2007). Once the blood flow velocity exceed the limits on the colour legend, 'colour inversion' occurs where the machine will continue to represent the velocity of blood but will do this in the opposite direction. In practice, this would mean that if the velocity of blood exceeds the brightest red in the colour legend, then the machine would promptly change this to the brightest blue and then darken as per the colour legend. This issue could be frequently encountered when measuring the blood flow velocity through the closing patent ductus arteriosus where high blood velocities occur. As a part of this assessment, the size of the patent ductus arteriosus is also measured. When aliasing occurs, the colour flow may appear more pronounced and

spill beyond the boundaries of the ductus. This could result in falsely over estimation of the size of the ductus. To reduce the chance of this occurring in the study, the following precautions are taken. The scale is increased so that the colour signal is within the ductus arteriosus and secondly the images are compared in 2–D and measurements of the ductus obtained in both 2–D and in colour. The advent of newer generation scanners equipped with harmonic imaging that improve spatial resolution and are proven to be superior to conventional echocardiography help in delineating the border of blood vessels and valves in 2–D (Caidahl et al. 1998).

1.9.5 Acoustic shadowing and reverberation

These challenges result from excessive reflection of sound before it reaches the transducer. Sound waves can pass through several tissues in the body of varied density. We know that further the sound waves travel, the poorer is the quality of the ultrasound image. The majority of tissues conduct sound waves well, but bone and air are known to cause marked reflection of sound waves. When ultrasound encounters these structures in its path, the resulting image quality becomes progressively poor with increasing depth. The primary reasons for this being the reflection and scattering of the ultrasound waves. The resulting loss in image quality is known as acoustic shadowing or attenuation artefact (Bulwer 2011). This artefact could potentially be encountered when measuring blood flow velocity in the superior mesenteric artery, which lies beneath gas filled intestines. The risk for this artefact is higher on day 3 for the more ‘mature’ preterm infants who are more likely to be on non-invasive respiratory support and be on milk feeds. Both of these factors contribute to gas within the bowels, which could cause reflection of the ultrasound waves rendering visualisation of the superior mesenteric artery difficult. The possible ways to reduce this would be to use lots of gel to ensure good contact between skin and the transducer. If air is present, repeating scan after a short period of time is helpful as the air pockets in the

bowels would have moved due to intestinal peristalsis.

The second artefact in this group is called reverberation artefact. This results from the to and fro reflections of sound waves between two highly reflective surfaces (Bulwer 2011) before it is detected by the transducer. The reflections typically appear distal to the reflective surface. When performing ultrasound scans of the heart, visualising extra structures in the heart further away from the transducer (Lawrence 2007) could be a consequence of this phenomenon in some cases. This could potentially mislead the sonographer to the presence of structural anomalies. Typical structures that could reflect waves in this fashion include the ribs and pericardium. Reverberations from the pleuro–pericardial surface could result in 'comet tail' artefacts. Reverberation artefacts as a whole are important to recognise due to the above-mentioned problem. The solution to this problem is to interrogate the heart using different echocardiographic views and confirming structural integrity before carrying out functional assessments.

1.10 Comparison between Doppler ultrasound with other methods available for estimating cerebral blood flow

Cerebral blood flow measured using the right common carotid artery (Sinha et al. 2006) in well preterm infants showed median (SD) value of 22.1 (7.3) ml/100gms/min which was comparable to 19.8 (5.3) ml/100gms/min using the ^{133}Xe clearance technique (Greisen 1986) in a similar group of infants. Doppler ultrasound has been used in measuring cerebral blood flow in both preterm and term infants who were breathing unaided (Sinha et al. 2006). This study used the right common carotid artery to estimate cerebral blood flow and concluded that 22% of the CO contributes to cerebral blood flow assuming that both carotid arteries contributed equally to cerebral blood flow. Using this method, cerebral

blood flow was measured to be about 20 ml/kg/min.

Cerebral blood flow in ventilated preterm infants is known to be reduced as compared to preterm infants who are not ventilated (Skov et al. 1991). Studies that used NIRS (Tyszczuk et al. 1998) to estimate cerebral blood, found the measured cerebral blood flow to be about 13.9 ml/100g/min in 14 infants who had a median gestation of 27 weeks. Cerebral blood flow estimation using Xe^{133} (Skov et al. 1991) found that the measured cerebral blood flow to be 14.8 ml/100g/min in a group of 16 infants, of whom 11 were mechanically ventilated. This study was conducted primarily to compare cerebral blood flow using NIRS and Xe^{133} found that cerebral blood flow values using Xe^{133} were comparable to those obtained by NIRS.

1.11 Measurement of superior mesenteric artery blood flow

The methods available for measurement of the superior mesenteric artery blood flow will be discussed in detail in this section.

1.11.1 Near Infrared spectroscopy

Near Infrared spectroscopy (NIRS) as previously described is a method that has been frequently used in the neonatal unit in premature newborn babies. It has been used primarily to study organ blood flow and some studies have used NIRS to investigate intestinal perfusion (Gillam-Krakauer et al. 2013) and as a marker for the development of necrotising enterocolitis (NEC) (Gay et al. 2011). It works on the principle of the relative transparency of intact biological tissue to light in the infrared spectrum, which allows for measurement of absorption of chromophores like oxyhaemoglobin and deoxyhaemoglobin. Though this

method is used widely to estimate blood flow in different parts of the body, it is not without its limitations. Some of the limitations which rules out this method to estimate mesenteric blood flow in my study include attenuation of the infrared light by intra-abdominal tissue, requirement of stable contact between the optical fibre and skin is crucial, disturbances from movement artefact secondary to ventilation and most importantly the risk of burns to the fragile skin due to prolonged contact are some of the technical challenges one could encounter in this group of babies.

1.11.2 Doppler ultrasonography

Doppler ultrasonography is a well-established technique to interrogate the intestinal blood supply (Leidig 1989, Murdoch et al. 2006). It has been extensively used in growth restricted premature infants to study the impact of feeding (Kempley et al. 2014) on intestinal blood supply and to identify infants at risk of NEC (Murdoch et al. 2006). The ease of performing scans at the bedside with minimum disturbance to the infant, ready availability of an ultrasound scanner in most neonatal units and most importantly the lower risk of injury to the fragile skin of babies in this gestation makes Doppler ultrasonography the ideal mode for investigating the intestinal blood flow in my study.

1.12 Comparison between Doppler ultrasound with other methods available for estimating superior mesenteric blood flow

Superior mesenteric blood flow measured using Doppler ultrasonography was compared with NIRS (Gillam-Krakauer et al. 2013). In infants between 25 to 31 weeks gestation with a median weight of 1200 grams, this study found a good correlation between superior mesenteric artery blood flow measured using NIRS and Doppler ultrasonography.

1.13 Electroencephalography in extremely preterm infants

1.13.1 Introduction

Advances in medicine over the last few decades have witnessed more preterm infants surviving. The majority of extremely preterm infants however remain at high risk of motor, cognitive, behavioural, emotional and academic challenges (Larroque et al. 2008, Johnson and Marlow 2017). Identifying preterm infants who are at risk of adverse neurodevelopment can help clinicians to target administration of neuro-protective measures to these babies. Some of these measures that are currently in use include antenatal administration of intravenous magnesium sulphate (Doyle et al. 2009) to the mother and postnatal administration of caffeine (Doyle et al. 2010) or erythropoietin (Neubauer et al. 2010) to the baby. The use of electroencephalography is well established in term infant with hypoxic ischaemic encephalopathy to diagnose seizure activity in the muscle relaxed infant, to study the evolution of the EEG activity; for example the emergence of sleep wake cycling, for comparison of electrical activity at different time points and to assist clinicians in predicting long term prognosis (Dunne et al. 2017).

The use of EEG, both as a research and clinical tool, has been extended to the extremely preterm infant. Studies have examined the practical challenges associated with measuring EEG in this group of infants (Lloyd et al. 2015). The work over the years in this group of infants have led to more normative EEG data existing now for the extremely preterm infant (Vecchierini et al. 2007) across different gestational ages. It is well known that abnormal EEG activity in preterm infants in associated with poor neurodevelopmental outcome (Tharp et al. 1989, Lacey et al. 1986, Hayashi-Kurahashi et al. 2012). EEG has

been used in preterm infants to predict complications like intraventricular haemorrhage (Clancy et al. 1984) and further work done on preterm infant EEG has shown that seizure activity is associated with adverse neurodevelopment (Shah et al. 2010).

EEG waveforms are generally classified according to their frequency, amplitude, shape and the site on the scalp from which they are recorded. The frequency of most brain waves range from 0.5 to 500 Hz. The majority of waves are classified in the descending order of frequency as beta, alpha, theta and delta waves (Sucholeiki, 2014).

Delta waves have a frequency of less than 3 Hz. They are classified as slow waves and seen in deep sleep in individuals of all age groups. Delta waves and sharp waves were the most common waveforms seen in the ictal period in the preterm infant (Patrizi et al. 2003).

1.13.2 Normative electroencephalographic data for extremely preterm infants

In this section, the various electroencephalographic parameters for infants born between 24 to 30 weeks gestation is discussed. For the purpose of this, I have sourced my data predominantly from the work done by Vecchierini and colleagues (Vecchierini et al. 2007). In the extremely preterm infant, EEG activity is characterised by discontinuity, lability and fragmentation. The more immature the infant, the more marked are these EEG aspects. The lack of agreement on the minimal time required for analysis and the minimal time to consider what constitutes discontinuous EEG makes the interpretation of EEG in preterm infants difficult.

I will discuss some of the terms used in conjunction with describing the EEG in preterm

infants before describing what changes are involved in the maturation of EEG parameters with increasing gestation.

A) Periods of electroencephalographic activity

Cerebral electrical activity duration increases with gestational age. In the more immature infant, cerebral electrical activity of high amplitude occurs in very short bursts of up to 1 second. In infants of 24 to 27 weeks gestation, the bursts of amplitude > 50 microvolts can occur between 1 and 83 seconds with a mean duration of 50 seconds. At 28 to 29 weeks gestation, the bursts of > 30 microvolts in two channels can last between 1.4 to 159 seconds. The longest duration of these could last up to 10 minutes. At 30 weeks gestation, there is good correlation between the behavioural state and EEG activity is acquired. Studies have demonstrated that an increase in the delta waves between day 1 and 4 is useful for prognosticating and is associated with a good neurodevelopmental outcome (Victor et al. 2005*a*).

B)Interburst Interval

Interburst interval is a period of electrographic quiescence that is present in all premature infants. The interburst interval shortens with increasing gestational age. In infants between 24 to 27 weeks gestation, it represents 45% of the tracing at a threshold of 15 microvolts. They could last between 23 to 60 seconds. The duration is usually less than a minute in babies of this gestation. At 28 to 29 weeks gestation, the IBI for a threshold of 30 microvolts is between 1.6 to 26.6 seconds and at 30 weeks gestation, discontinuity is almost exclusively seen during ‘quiet sleep’ and the duration is less than 20 seconds.

In infants between 24 to 27 weeks gestation, delta waves occurring over the temporal

region tends to be unilateral with right predominance. Delta waves are also seen bilaterally over the occipital and less frequently over the frontal regions. In infants between 24 to 25 weeks gestation, the frontal region has short bursts of high voltage slow waves, which disappear by 28 weeks gestation (Vecchierini et al. 2007). At 28 weeks, the amplitude of the delta waves is between 30 to 300 microvolts and frequency of 0.5 to 2 Hz. There is a progressive displacement of delta waves to the centro-occipital area with maturation of the infant.

Theta waves occur across all gestations. At 24 weeks, bursts of theta waves occur diffusely or over the temporal regions. They become more abundant over 26 to 27 weeks gestation. By 28 to 29 weeks, theta waves predominate over the temporal regions. Theta waves could originate in the occipital regions and finally migrate towards the temporal region (Vecchierini et al. 2007).

1.14 Relationship between blood pressure and various clinical parameters in extremely preterm infants

1.14.1 Relationship between blood pressure and cardiac output

Blood pressure is dependent on the blood flow (CO) and the systemic vascular resistance. CO, in turn, as I alluded to earlier is the product of the heart rate and the stroke volume. Several cardiovascular studies have examined this relationship.

Kluckow et al (Evans and Kluckow 1996) studied 67 preterm infants who had a median gestation of 28 weeks and 1015 grams respectively. They compared the BP and CO in

these infants who required mechanical ventilation. This study found a poor correlation between BP and CO ($r = 0.14$). However, the relation was stronger ($r = 0.38$, $p = 0.001$) when they only included infants whose PDA were closed and those with insignificant PDA, which was defined as PDA with less than 1.5 mm diameter with no evidence of ductal, steal. Infants who had PDA were more likely to have a larger CO due to the shunting across the ductus arteriosus. PDA, in addition to affecting systemic flow, exposes the left ventricle to the combined pulmonary and systemic vascular resistance. PDA is also associated with increased prostacyclin, which in turn causes vasodilation (Kluckow et al. 1999, Hammerman et al. 1986). They found that 27% of infants with a normal BP had a low CO defined as being less than 150 ml/kg/min. Up to 25% of infants with a low BP had a normal CO. Higher mean airway pressure and large ductal diameter were found to have a significant negative influence on the BP in this study. Higher gestational age and left ventricular output were found to have a significant positive influence on the BP in this study using stepwise regression.

Lopez et al (Lopez et al. 1997) compared 80 infants less than 34 weeks with a birth weight below 1750 grams with respiratory distress syndrome requiring inotropic support to infants with respiratory distress syndrome who were normotensive. The aim of this study was to determine the incidence of supranormal haemodynamics in infants with and without inotropic support. They compared CO, stroke volume, systemic vascular resistance, heart rate and mean arterial BP between different groups of infants based on the inotropic requirement. This study found that infants on dopamine and dobutamine had supranormal CO, low systemic vascular resistance and reduced mean arterial BP. This study illustrates the weakness in the correlation between the BP and CO but highlights the importance of other factors mainly the systemic vascular resistance that plays an important role in addi-

tion to the respiratory support in determining the BP. The weakness in the relation between BP and CO was also demonstrated by Rozé et al (Rozé et al. 1993) who compared the response of dobutamine and dopamine in the hypotensive preterm infant.

It is clear from the above studies that there is a poor correlation between BP and CO. The effect of the systemic peripheral vascular resistance, patent ductus arteriosus and respiratory support that is required in these infants play a role in the lack of correlation.

1.14.2 Relationship between circulatory parameters and markers of peripheral perfusion

The most frequently used markers of peripheral perfusion in clinical practice include heart rate, capillary refill time, serum lactate, urinary output, skin colour (de Boode 2010).

Heart rate

Heart rate influences CO. As a compensatory mechanism for low CO, one would expect the heart rate to increase. This would be the ideal way to increase the CO in preterm infants because of the limited ability of the preterm myocardium to compensate for reasons explained earlier. However, the ability to normalise CO can only be achieved if the end diastolic volume is preserved. As the coronaries are supplied during diastole, a very high rate can adversely affect perfusion to the myocardium. One would expect that a normal heart rate would mean normal CO and therefore and normal haemodynamic status. However, few studies that examined the heart rate in infants with low BP and normal BP did not show a significant difference in the heart rate between the two groups (Kluckow and Evans 2000*a*, Gill and Weindling 1993). Moreover, other factors such as inotropes, temperature, hydration, response to painful stimuli could all elevate the heart rate. A large increase in heart rate which is sustained will help to tease out these issues and may point towards to a low systemic flow and therefore CO.

Capillary refill time

Osborn and colleagues (Osborn 2004) examined capillary refill time with central and peripheral temperature difference as surrogate markers for detecting low flow states in 128 infants born less than 30 weeks gestation. SVC flow was measured using echocardiography. There was a significant negative correlation between SVC flow with central and peripheral capillary refill time. They found that a CRT $\geq$3 had a 55% sensitivity and 81% specificity for detecting low flow states. Further work by Miletin et al (Miletin et al. 2009) who studied 38 infants using SVC flow and capillary refill time found a poor correlation between the two. They concluded that a prolonged capillary refill time of > 4 seconds and serum lactate of > 4 mmol/L had a specificity of 97% for detecting low flow states. Wodey et al (Wodey et al. 1998) studied 100 infants and concluded that there was a significant negative correlation between cardiac index and capillary refill time. LeFlore and colleagues (LeFlore and Engle 2005) examined capillary refill time in the first 4 hours after birth in 42 term infants and compared it with non–invasive BP. They found a direct relation between the two parameters.

Lactate

Lactate, a product of anaerobic metabolism, is thought to be produced in the tissues when there is a reduction/ lack of adequate systemic flow to meet the metabolic demands. Hence a raised lactate level is used as a surrogate marker for reduced CO. Blood lactate levels of < 2.5 mmol/L are considered to be normal and raised lactate levels (> 2.5 mmol/L) have been shown to be associated with an increased mortality (Deshpande and Platt 1997). Other blood gas parameters such as pH and base excess were poor predictors of lactate. Miletin et al (Miletin et al. 2009) examined 38 infants using SVC flow and capillary refill time found a poor correlation between the two. They concluded that a prolonged capillary refill time of > 4 seconds and lactate of > 4 mmol/L had a specificity of 97% for detecting

low flow states.

Urine output

Urine output increases in the first few days of life which reflects increased glomerular filtration rate and therefore increased perfusion to the kidneys. Subsequent reduction in the urine output could be related to poor fluid intake (Bidiwala et al. 1988). Physiologically, one would expect to see a positive correlation between CO and urine output. However, in preterm infants, it should be noted that the immature renal tubule is incapable of concentrating urine. So in the face of a high serum osmolality, preterm infants may not be able to reduce urine output (Linshaw 1998).

1.15 Relationship between electroencephalography and various clinical parameters in extremely preterm infants

1.15.1 Relationship between electroencephalography to cardiac output and cerebral blood flow

The relationship between electroencephalography and cerebral blood flow in extremely preterm infants has been explored by some studies, which have significant heterogeneity between them. In the next few paragraphs, I have discussed four studies examining this relationship.

Shah et al (Shah et al. 2013) studied 92 infants who had a median gestation and birth weight of 26 weeks and 882 grams respectively. A mixture of invasive and non-invasive BP was used for analysis. A 2-channel EEG was used for recording electroencephalography. SVC flow and RVO output were used as surrogate markers for cerebral blood flow and CO respectively. There was a significant linear relation between aEEG amplitude and

SVC flow at 12 hours of age only. There was no association between SVC flow and aEEG found in this study at 24 or 48 hours of age after adjusting for gestational age and severity of illness. This study also found that infants who received inotropes for hypotension were noted to have lower aEEG amplitude and continuity at 12, 24 and 48 hours of age respectively. These differences persisted even for infants whose cardiovascular parameters were normal within 12 hours of age. The strengths of this study include the large number of subjects. However, the limitations of this study include the use of a mixture of invasive and non-invasive BP data, lack of published data on serum carbon dioxide levels which could influence cerebral blood flow and lastly the use of superior vena cava as a surrogate of cerebral blood flow. Estimation of cerebral blood flow using the superior vena cava has a couple of limitations; firstly the superior vena cava is cresentric in shape. Therefore using the same methodology to calculate the area of the vessel as you would for a circular vessel, is prone to error and inaccurate results. Secondly, the superior vena cava receives drainage from the brachiocephalic vein which drains the upper limbs which could overestimate cerebral blood flow.

Victor et al (Victor, Appleton, Beirne, Marson and Weindling 2006) studied 40 infants who had a median gestation and birth weight of 27 weeks and 927 grams respectively. EEG was measured using six electrodes for a period of 75 min on each day for the first four days. A 60-minute artefact free period was used for analysis. This study used near infrared spectroscopy to measure peripheral blood flow and cerebral fraction oxygen extraction as a marker of cerebral blood flow. Invasive mean BP was measured every 4 minutes and right and left ventricular outputs were measured. This study found no relationship between LVO, RVO, peripheral blood flow and the indicators of cerebral perfusion. EEG was found to be normal with reduced RVO, LVO and mean BP of more than 30

mmHg. However, some of the limitations of this study were that all infants weighing less than 1250 grams were treated with indomethacin. The effect of indomethacin on cerebral blood flow is not well documented. The other limitation is the use of M-mode to measure the diameter of the aortic valve. The downward motion of the aortic valve in systole could result in underestimating the diameter and result in a falsely low left ventricular output. BP measurements were recorded every 4 minutes, hence subtle changes in BP could be missed with such large intervals.

West et al (West et al. 2006) studied 40 infant who had a median gestation and birth weight of 27 weeks and 945 grams respectively. All infants had SVC flow, RVO, BP measured and EEG recorded. SVC flow were performed close to 5, 12, 24 and 48 hours old and coinciding with EEG where possible. RVO was measured using parasternal short axis or tilted parasternal long axis. The diameter was measured at the hinge points of the pulmonary valve. Invasive BP was available in 2/3rd of the infants and recordings downloaded every 1 min. BP readings averaged over the duration of echo. Oscillometry was performed if no BP was recorded within an hour of echo. EEG was recorded 2 to 4 times (2 to 12 hour periods) during the first week of life using BRM monitor. Median and minimum EEG amplitude along with EEG continuity were analysed for 60 min before and after echocardiography. SVC flow was positively related to EEG continuity only at 5 hours and not at any other time period. RVO measured 12 hours after birth was related to EEG amplitude and continuity at 12 to 24 hours after birth. Mean arterial BP and diastolic BP at 12 to 24 hours were also related to EEG continuity at 12 to 24 hours. Both low flow and low BP have an immediate and prolonged impact on EEG activity. The main limitations of this study were that it was a combination of 2 studies –one was to assess haemodynamic changes and the other looking at EEG changes over a period of time. At the various dif-

ferent time points babies who had assessments varied from 24 to 31 babies and lastly a mixture of non-invasive and invasive BP analysed together.

Greisen et al (Greisen et al. 1988) studied 24 infants with a median gestation of 28.5 weeks. The aim of this study was to examine the relation of arterial BP, cerebral perfusion and neural function to the expansion of the intravascular volume by transfusion in preterm infants during the first day of life. The various parameters measured included aEEG, internal carotid artery blood flow velocity using sonography and mean arterial BP. aEEG measured was commenced at a median age of 3 hours and continued for 24 hours. In eight out of the twelve infants who had complete data, transfusion was followed by an increase in the BP and internal carotid artery blood flow. In two babies, BP increased but not internal carotid artery blood flow. The main limitation of this study includes the small number of babies that were studied and the routine use of phenobarbitone and diazepam, which could influence the EEG.

In summary, Shah et al showed that there was a linear relationship between electroencephalography and SVC flow in the first 12 hours of age only with no association found at 24 or 48 hours of age. West et al found similar finings at 5 hours of age only. However, these findings were different from that of Victor et al who found that EEG remained normal with low LVO and RVO. The main drawback of these comparisons is the heterogeneity between the various studies that were compared here. The real relationship between electroencephalography and cerebral blood flow should be answered through a randomised controlled trial.

1.15.2 Relationship between electroencephalography and blood pressure

Victor et al (Victor, Appleton, Beirne, Marson and Weindling 2006) found electroencephalography was normal with a mean BP of greater than 30 mmHg. Shah et al (Shah et al. 2013) studied 92 infants using a mixture of invasive and non invasive BP during the first 48 hours of life and found no significant relation between electroencephalography and BP or CO. However, they found that infants who received inotropes had significantly lower amplitude in the first 12 hours of age. West et al (West et al. 2006) studied 40 infants and found an association with BP and continuity of the electroencephalogram in the first 12 to 24 hours. They also found that infants who were in the lowest quartile for CO and BP had lower EEG continuity. Further work by Victor et al (Victor, Marson, Appleton, Beirne and Weindling 2006) examined BP threshold level below which there was reduced electrocortical activity. Having done studies on 35 low birth weight infants who were less than 30 weeks gestation, they found that 4 infants were noted to have lower EEG amplitude and longer interburst intervals at a mean BP below 23 mmHg. They concluded that EEG remained normal above a mean BP of 23 mmHg.

1.15.3 Relationship between electroencephalography and blood gas parameters

Several studies have compared changes in EEG with various biochemical parameters (Victor et al. 2005*a*, Wikstrom et al. 2011, Granot et al. 2012, Victor et al. 2014). Human and animal studies have shown that serum carbon dioxide levels influence cerebral blood flow such that an increase in carbon dioxide results in cerebral vasodilatation and decrease in carbon dioxide levels causes a reduction in cerebral blood flow secondary to vasoconstriction of the cerebral vasculature (Pryds et al. 1990, Koons et al. 1993, Liem et al. 1995, Nakahata et al. 2003, Giller 1993) . Varying levels of carbon dioxide affect cerebral blood

flow by predominantly causing change in the diameter of the blood vessels, which in turn affects cerebral blood flow velocity and volume. Victor et al (Victor et al. 2005*a*) demonstrated that a reduction in $PaCO_2$ levels led to an increase in the delta band of the EEG and a decrease in the beta and alpha bands suggesting a slowing of the EEG. In addition, cerebral fractional oxygen extraction, a surrogate marker for cerebral blood flow was also measured. One would normally expect cerebral fractional oxygen extraction to increase in response to reduced cerebral blood flow which was demonstrated in this study. Hypocarbia resulted in slowing of EEG in this study can be explained by two main mechanisms; firstly, hypocarbia induced vasoconstriction resulting in reduced cerebral blood flow and secondly hypocarbia leads to left shift in the oxyhaemoglobin dissociation curve which results in increased affinity and therefore making it harder for oxygen to be released to the tissues. Hypercarbia has also been found to suppress EEG (Niedermeyer and da Silva 2005, Martoft et al. 2002) by increasing the interburst interval. This predominant action of carbon dioxide is mediated by altering the membrane potential. High levels of dissolved carbon dioxide in the serum results in increased diffusion of carbon dioxide across the cell membrane, which increases intracellular hydrogen ions. The resultant increase in hydrogen ions alters the Na: K ratio and the membrane potential (Meyer 1961). An increase in the carbon dioxide levels also shifts the oxyhaemoglobin dissociation curve to the right resulting in oxygen being more readily delivered to the tissues.

The carbon dioxide induced changes in electroencephalography described above were more predominant on day 1 of life as opposed to day 3 of life (Victor et al. 2005*a*) in human studies. The changes in cerebral haemodynamics in the immediate postnatal period help us to understand why this happens. We know from previous work in humans that CO (M. Kluckow 1996), BP (Cunningham et al. 1999) and cerebral blood flow (Meek et al.

1998) increases between day 1 and day 3 of life. As cerebral perfusion is at the lowest on day 1 of life, infants are most vulnerable at this stage. We also know that cerebral vasoreactivity increases in the first few days of life. This could explain why the electroencephalogram was more sensitive to changes in carbon dioxide on day 1 rather than day 3 of life when the various cerebrovascular parameters have increased. BP also has been found to play a role in the reactivity of the cerebral blood flow to carbon dioxide. Jayasinghe et al (Jayasinghe et al. 2003) demonstrated that normotensive infants had an intact cerebral autoregulation and diminished response to arterial carbon dioxide. In contrast, hypotensive infants were noted to have altered or absent autoregulation with no response to varying arterial carbon dioxide levels.

1.15.4 Relationship between electroencephalography and morphine used in the neonatal intensive care unit

Opiates are the most commonly used analgesic in the neonatal intensive care unit. Among opiates, morphine is the most frequently used analgesic in the neonatal intensive care unit (Hall et al. 2007). Morphine has a slower onset of action that other opioids such as Fentanyl because of its lower lipid solubility. The mean onset of action is 5 min and peaks at 15 minutes. Morphine, in the liver, is broken down into two main compounds namely morphine-3-glucronide and morphine-6-glucronide. Morphine-3-glucronide is a potent opioid antagonist whereas morphine-6-glucronide is a potent analgesic. In preterm infants, due to liver immaturity and reduced amounts of UDP-glucuronyl transferase, morphine is broken down to predominantly morphine-3-glucronide. This accounts for the tolerance that is noted in these infants 3 to 4 days after starting morphine infusion.

The effect of morphine on other systems in the preterm infant has been well documented. Randomised controlled trials have shown that morphine infusions and additional bolus

doses of morphine were associated with hypotension in infants between 23 to 26 weeks gestation in and those with pre-existing hypotension (Hall et al. 2005). A randomised, double–blind, placebo–controlled trial carried out between 2000 to 2002 studied 150 infants (morphine, n=73 and placebo, n=77) with a median gestation of 29 weeks. Infants on morphine infusion had a significantly lower incidence of intraventricular haemorrhage (23% vs. 40%, p=0.04), but there was no statistically significant difference in the long term neurodevelopmental outcome (10% vs. 16%, p=0.66) (Simons et al. 2003). Further randomised placebo–controlled trials involving 898 infants (morphine, n=449 and placebo, n=449) born at 23–32 weeks demonstrated that there was a prolonged need for ventilator support and increased time to achieve feeds in infants who received morphine infusions (Anand et al. 2004).

The effects of morphine infusion on the electroencephalography of preterm infants of varying gestational ages have been well documented. Natalucci et al (Natalucci et al. 2014) who studied 96 infants with a mean gestation of 29 weeks reported that morphine administration affected aEEG amplitude and maturity. Further work by Norman et al (Norman et al. 2013) who enrolled 34 infants with a mean gestation of 26 weeks in a randomised controlled trial compared the use of premedication (rapid sequence intubation using thiopental with remifentantil was compared with morphine) for endotracheal intubation. This study showed that infants who received rapid sequence intubation had EEG depression lasting for less than 3 hours. In contrast, infants who received morphine as premedication were found to have more discontinuous EEG with less developed cyclicity for a period of 24 hours. Moreover, in the first 9 hours after administration, the interburst interval was found to be much higher in infants who received morphine. It is noteworthy that the mean arterial BP between the two groups were not different. Further studies

examining 56 preterm infants with a median gestation of 30 weeks have shown that morphine administration was associated with reduced continuity (Herbertz et al. 2006) and prolonged periods of electrical quiescence with even epileptiform activity, which resolved when morphine was stopped (n=20, median gestation of 34 weeks, range 26–41 weeks) (Young and da Silva 2000). Bell et al (Bell et al. 1993) retrospectively studied 18 infants with a mean gestation of 29 weeks who received morphine. They found that these infants had prolonged interburst intervals. They also found that infants who received a single dose of diazepam had a marked additive effect on the EEG depression caused by the baseline sedative effect of morphine. This depressive effect was prolonged for a period of up to 12 hours. Da Silva et al (da Silva et al. 1999) published a case reports of infants who had burst suppression on EEG whilst on morphine which reversed upon administration of naloxone. It is evident from various studies that morphine causes a depressive effect on the electroencephalography of preterm infants.

1.16 Effects of various inotropic agents on the cardiac output, cerebral blood flow, gut blood flow and electroencephalography.

There is much debate regarding the appropriate choice of inotrope for the treatment of low BP in the neonatal intensive care unit. A wide variation exists in practice among different clinicians even within the same neonatal unit. However, with the increasing use of functional echocardiography, this trend is slowly changing with more and more clinicians now using a combination of clinical and echocardiographic assessment to guide the inotrope of choice (Gupta and Donn 2014).

In order to maintain a standard approach among various clinicians in this study, we used our own unit guideline (section 7.5) for starting inotropic agents for any of the infants recruited into the study. In this section, my aim is to explore the effects of commonly used inotropes, using published pharmacological and clinical studies, on the various physiological parameters that will been measured in this study, understand how these inotropes affect each of the parameters measured and consider any significant effects when analysing the results of this study.

The inotropic agents used in the neonatal intensive care unit can be classed broadly under catecholamine's and sympathomimetic drugs. As a group, these drugs affect different systems to a varying degree and their effects can be divided into seven main categories outlined below (Westfall and Westfall 2006).

i. Peripheral excitatory action on certain smooth cells (for example; blood vessels of the skin, kidney and mucous membranes) and the gland cells (for example; the salivary and sweat glands.

ii. Peripheral inhibitory action on certain smooth cells (for example; those supplying the intestinal wall, bronchial tree and blood vessels supplying the skeletal muscles)

iii. Cardiac excitatory action that leads to increased heart rate (chronotropic action) and increased force of contraction (inotropic action)

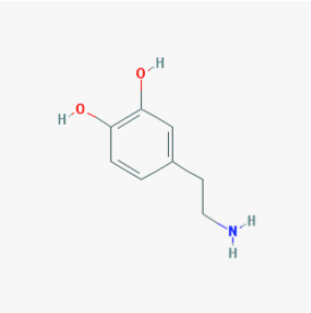
iv. Metabolic actions which lead to increased glycogenolysis in the liver and muscle and increased release of free fatty acids from adipose tissue

v. Endocrine actions such as increasing or decreasing the secretion of insulin, renin and pituitary hormones

vi. Central nervous system actions such as increased wakefulness and psychomotor activity and reduced appetite

vii. Prejunctional actions that lead to release or more importantly inhibition of neuro-transmitters.

1.16.1 Dopamine



Chemical structure of Dopamine *Image courtesy –PubChem*

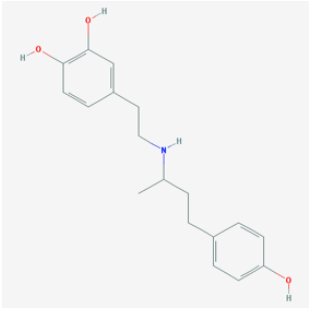
Dopamine (3,4-dihydroxyphenylethylamine) is an immediate metabolic precursor of adrenaline and noradrenaline. It possesses important pharmacologic properties and is produced centrally predominantly in the substantia nigra where it regulates locomotion, emotions and cognition. It exerts its effects peripherally on the epithelial cells of the proximal tubule, vasculature and pituitary where it has diuretic properties, affects vasomotor tone and hormone secretion respectively (Westfall and Westfall 2006).

Dopamine receptors are classified into two main types; D1 like receptors and D2 like receptors. D1 like receptors has 2 subtypes —D1 and D5, which activate adenylyl cyclase, and D2 like receptors, which has 3 subtypes —D2, D3 and D4 that inhibit adenylyl cyclase and activate potassium channels (Missale et al. 1998). Dopamine falls into the category of mixed adrenergic agonists, exerting its action by directly stimulating the receptors and indirectly by releasing agents that stimulate the receptors.

The various effects of dopamine on the tissues depend on the affinity of the various dopaminergic receptors to dopamine. At low doses, dopamine stimulates the D1 receptors in the renal, mesenteric and coronary beds leading to stimulation of adenylyl cyclase, which increases intracellular levels of cyclic AMP levels resulting in vasodilatation. However, at higher doses dopamine has an inotropic effect by stimulating the β1 adrenergic receptors in the myocardium. Dopamine, being a mixed adrenergic agent, also causes the release of noradrenaline from the nerve terminals that contribute to the inotropic effect. At low or intermediate dosing, dopamine does not increase the peripheral vascular resistance. At higher doses, dopamine activates the α1 adrenergic receptors resulting in more generalised vascular vasoconstriction.

In the newborn infant several studies (Osborn et al. 2002, Greenough and Emery 1993, Rozé et al. 1993, Subhedar and Shaw 2000) have demonstrated that dopamine when compared to dobutamine was more effective in increasing the BP and treating hypotension. This effect is primarily by increasing the peripheral vascular resistance. Studies have also demonstrated that there is a poor correlation between the plasma concentration of dopamine and the response to BP (Bhatt-Mehta et al. 1991). Dopamine at low doses of 2 microgram/kg/min produced diuresis and natriuresis, which was a result of increased organ blood flow (Seri et al. 1993) but this vasodilatory effect of dopamine at low doses is debated (Barrington 1995). As far as cerebral blood flow is concerned, there remains uncertainty as to whether there is an increase in cerebral blood flow in infants receiving dopamine (Munro et al. 2004) or if cerebral blood flow is unaffected by dopamine (Seri et al. 1998, Lundstrøm et al. 2000). The endocrine effects of dopamine were also studied and it was found to produce reversible reduction of TSH, T4, prolactin and growth hormone (Filippi et al. 2007).

Dopamine in clinical practice has been typically used up to a maximum dose of 20 microgram/kg/min. There have been concerns raised regarding excessive stimulation of the α1 adrenergic receptors at doses beyond 20 microgram/kg/min, leading to potent vasoconstriction of the renal blood vessels resulting in oliguria and increasing after load. Studies that have shown that at high doses of dopamine, there is no effect on the urine output in newborn infants (Perez et al. 1986). Further work (Repetto et al. 1999) using dopamine doses of between 24 to 250 microgram/kg/min found no reduction in the urine output, improved oxygenation index with no impairment to pulmonary blood flow and concluded that no adverse effects were found at such high doses. Seri et al (Seri and Evans 1998) investigated the effect of adrenaline on the urine output of infants who were already receiving a high dose of dopamine (up to 26 micrograms/kg/min) and concluded that the addition of adrenaline improved the BP and did not induce oliguria. The main reason proposed for the lack of oliguria is the down regulation of α1 adrenergic receptors that occurs in disease states in these infants. Manouchehri and colleagues (Manouchehri et al. 2013) compared the effects of using high dose dopamine (> 20 micrograms/kg/min) versus a combination of dopamine and epinephrine in asphyxiated swine models and found similar increases in BP and cardiac performance. There was no change in the common carotid vascular resistance but they found the mesenteric vascular resistance to be significantly decreased in the group that received high dose dopamine. Further randomised controlled trials (Barrington et al. 1995) comparing dopamine and adrenaline using piglet models concluded that dopamine at doses of 32 micrograms/kg/min did not have a significant effect on the hepatic blood flow, portal venous flow and mesenteric vascular resistance.



Chemical structure of Dobutamine *Image courtesy –PubChem*

1.16.2 Dobutamine

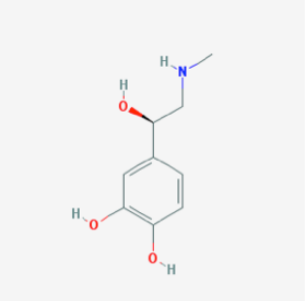
Dobutamine has a similar chemical structure to dopamine but possesses a bulky aromatic substituent in the amino group. Dobutamine has been classed, from a practical point of view, as a β agonist. However, commercial available dobutamine is a racemic mixture of two enantiomers (+/-). The (+)–enantiomer of dobutamine predominantly stimulate the β1 and β2 adrenoceptor receptors whereas the (-)–dobutamine stimulates the α1 adrenoceptor receptors (Ruffolo and Messick 1985*a*,*b*). Dobutamine has been used routinely in cardiac failure wherein it stimulates the β1 and α1 adrenergic receptors in the myocardium resulting in increased myocardial contractility, augmented stroke volume and improved CO. It has a predominant inotropic action and less of a chronotropic action. It also causes a reflex withdrawal of the sympathetic tone of the peripheral vasculature leading to vasodilatation. In the peripheral blood vessels, the β2 adrenergic receptor activity of dobutamine inducing vasodilation is offset by the α1 adrenergic vasoconstrictor activity of dobutamine thus causing no significant change in the BP (Ruffolo 1987).

Dobutamine is more effective in improving the left ventricular output and hence recommended for use in instances of poor myocardial contractility secondary to hypoxic insult or septic shock (Martinez et al. 1992). In premature newborn infants, dobutamine has been shown to improve CO to a greater degree than dopamine (Osborn et al. 2002). A

Cochrane review investigating dopamine and dobutamine for treatment of hypotension in preterm infants (Subhedar and Shaw 2003) concluded that there was no difference between dopamine and dobutamine as far as neonatal mortality and cranial ultrasound abnormalities like periventricular leukomalacia and intraventricular haemorrhage were concerned.

The optimal dosing for dobutamine remains uncertain. There is a wide variation in the pharmacokinetics between individuals for a given dose of dobutamine. Studies in term and unstable preterm infants have shown that dobutamine when used at a dose between 7.5 microgram/kg/min to 10 microgram/kg/min was effective in increasing CO by improving myocardial contractility (Stopfkuchen et al. 1987, 1990). A further study of 20 infants who had a mean gestation of 29 weeks found that dobutamine improved stroke volume and CO 20 minutes after commencing the infusion and there was sustained increase in blood flow to the anterior cerebral artery, superior mesenteric artery and the renal arteries that lasted for 8 to 10 hours after administration of the drug (Robel-Tillig et al. 2007). Dobutamine, unlike dopamine has been found to have no effect on endocrine functions of TSH, T4, prolactin and growth hormone (Filippi et al. 2007).

1.16.3 Adrenaline



Chemical structure of Adrenaline *Image courtesy –PubChem*

Adrenergic agonists include three main agents, which are collectively called catecholamines. Adrenaline is a major hormone of the adrenal medulla. The precursor to adrenaline is no-

radrenaline, which is the principal transmitter of most sympathetic postganglionic fibres in the central nervous system and finally its precursor; dopamine is the predominant neurotransmitter of the extrapyramidal, mesolimbic and mesocortical pathways (Westfall and Westfall 2006). The precursor to dopamine is dopa and finally the precursor to dopa is the non-essential amino acid tyrosine.

Adrenaline (epinephrine) is a directly acting non-selective adrenergic agonist that potently stimulates both the α and β receptors. It has prominent actions on the myocardium, vascular and other smooth muscle. Adrenaline is one of the most potent vasopressor drugs known. It causes a much higher increase in the systolic BP than the diastolic BP, which results in an increase in the pulse pressure. The mechanism of increase in BP is mediated through 3 main routes; firstly through the potent inotropic effect, secondly via chronotropic effect and lastly through vasoconstriction of many vascular beds like the skin, mucosa, kidney and the veins (Westfall and Westfall 2006). At low doses, adrenaline has a predominant inotropic, chronotropic effect with vasodilatory effect on the pulmonary and systemic circulation.

Adrenaline, in addition to the predominant cardiovascular effects, also has significant metabolic effects. It will well known that skeletal muscles is a source of lactic acid in the human body (Consoli et al. 1990, Qvisth et al. 2007). Adrenaline has been found to induce release of lactic acid from the striated muscles, induces lipolysis and hyperglycaemia by inhibiting insulin stimulated glucose uptake at the cellular level (Gjedsted et al. 2011, Sherwin and Sacca 1984).

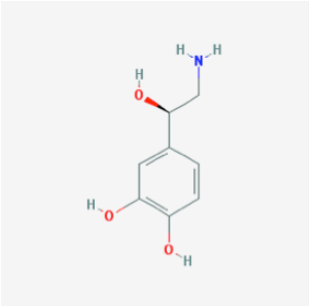
Adrenaline has been used for several years to treat hypotension and pulmonary hypoten-

sion in newborn infants (Zaritsky and Chernow 1984). Adrenaline exerts a different action when given in higher doses. Randomized studies in animal models have shown that at lower doses of between 200 to 800 nanograms/kg/min, adrenaline had vasodilatory effects on the pulmonary artery, but at doses greater than 800 nanograms/kg/min of adrenaline, the systemic arterial BP achieved was well above the pulmonary arterial pressures (Barrington et al. 1995). Studies using piglet models compared the effects on systemic arterial pressure, pulmonary arterial pressure, cardiac index, hepatic, portal and mesenteric blood flow when adrenaline and dopamine were given at varying doses –2, 10 and 32 micrograms/kg/min of dopamine and 200, 1000 and 3200 nanograms/kg/min of adrenaline respectively. This study found infusing dopamine at 32 micrograms/kg/min and adrenaline at a dose of 1000 nanograms/kg/min and 3200 nanograms/kg/min increased the systemic arterial pressure, pulmonary arterial pressure and cardiac index. Dopamine, at such a high dose, did not have a significant effect on the hepatic blood flow, portal venous flow and mesenteric vascular resistance. However, adrenaline at a dose of 3200 nanograms/kg/min decreased portal venous blood flow, total hepatic blood flow and hepatic oxygen delivery but increased mesenteric vascular resistance. There was also a significant rise in plasma lactate levels with this dose of adrenaline. Valverde et al (Valverde et al., 2006) compared the use of low dose adrenaline and dopamine in premature infants. They found that adrenaline produced a greater increase in heart rate, increase in plasma lactate within 36 hours of commencing the infusion, lower base excess requiring more bicarbonate infusions, higher glucose levels requiring insulin when compared to infants who received dopamine. More recent work on piglet models (Nachar et al. 2011) has shown that low dose adrenaline increases BP, systemic and regional blood flow to various organs. However, at high doses there was a reduction in systemic blood flow, increase in muscle blood flow, plasma glucose and lactate levels. Cerebral blood flow was not affected at either

dosage.

Adrenaline, being a potent vasoconstrictor, is not without its side effects. Inadvertent infusions of large amounts of adrenaline could lead to increase in the total peripheral vascular resistance causing a reduction in the CO inspite of maintaining a normal BP, tachycardia and severe tissue necrosis in the event of extravasation (Seri 2001).

1.16.4 Noradrenaline



Chemical structure of Noradrenaline *Image courtesy –PubChem*

Noradrenaline is a directly acting non-selective adrenergic agonist. Its chemical structure is very similar to adrenaline except for the lack of a methyl side chain in the amino group. It contributes up to 20% of the catecholamines from the adrenal medulla. It stimulates both the α and β receptors (α1, α2 and β1 receptors). It has relatively little action on β2 receptors, which are responsible for vasodilatation. Due to this reason, noradrenaline produces much more vasoconstriction when compared to dopamine and adrenaline. Noradrenaline also possess similar metabolic effects similar to adrenaline but only at very high doses. It produces hyperglycaemia and a rise in lactic acid similar to adrenaline.

Noradrenaline has been used in treating hypotension as a second agent, in addition to dopamine in preterm infants (Derleth 1997). Study in fetal lambs have shown that noradrenaline decrease basal pulmonary vascular tone (Jaillard et al. 2001) and improves pulmonary blood flow (Magnenant et al. 2003) by inducing pulmonary vasorelaxation

mediated via α2 receptors. The surge in noradrenaline at the time of birth is thought to produce pulmonary vasodilatation and reduce the pulmonary vascular resistance. There is a paucity of studies examining the use of noradrenaline in preterm infants. Tourneux and colleagues (Tourneux, Rakza, Abazine, Krim and Storme 2008) studied twenty-two term infants retrospectively and found that noradrenaline was effective in increasing BP, improving urine output and reducing lactate levels, thus suggesting that it improved CO and tissue perfusion. Term infants with cardiac dysfunction secondary to pulmonary hypertension were also found to have reduced ratio of pulmonary to systemic pressure, increased pulmonary blood flow increase and improved cardiac performance when treated with noradrenaline (Tourneux, Rakza, Bouissou, Krim and Storme 2008).

In addition to the cardiovascular benefits of noradrenaline, work on rats has shown that noradrenaline also possesses neuroprotective properties, mediated in part by induction and release of astrocyte monocyte chemoattractant protein 1 (Madrigal et al. 2009). Additional work in rat models by Landers et al (Landers and Sullivan 1999) showed that somatosensory conditioning requires noradrenaline.

Noradrenaline, like other inotropes, is not without side effects. Commonly seen side effects include tachycardia. A marked increase in the peripheral vascular resistance causing vasoconstriction can occur with inadvertent excessive administration. In the event of an extravasation injury, there is an increased risk of tissue necrosis and gangrene.

There is a paucity of data on the effects of different inotropic agents on the electroencephalography in premature newborn infant. Studies (Shah et al. 2013, Eaton et al. 1994*a*) have demonstrated that preterm infants who were on inotropic agents were found to have

a suppressed electroencephalogram. The exact reason for this is unclear. Speculations include a physiological insult at birth causing myocardial dysfunction resulting in reduced cerebral blood flow and abnormal neuronal activity. It is unclear if this suppression of the neuronal activity is secondary to the effect of inotropic agents or if it is simply a delay in the electroencephalography recovering from the initial insult.

Target	Important locations	Main actions
Receptors		
α1	Arterioles	Constriction
α2	Arterioles: mainly coronary and renal	Constriction
β1	Conducting system of heart	Increase in heart rate
	Atrial and ventricular muscle	Increase in contractility
	Arterioles in heart and skeletal muscle	Vasodilatation
β2	Conducting system of heart	Increase in heart rate
	Atrial and ventricular muscle	Increase in contractility
	Arterioles in heart and skeletal muscle	Vasodilatation
D1	Postsynaptic receptor in peripheral vasculature	Vasodilatation
D2	Presynaptic receptor in peripheral vasculature	Vasodilatation
Vasopressin ADH-R		
Enzymes		
Phosphodiesterase type III	All over	Inhibition of PDE III causes vasodilation and increased contractility

Table 1.3: Summary of targets for inotropes

Table reproduced with permission from Which inotrope and when in neonatal and paediatric intensive care? by M. A. Turner and P. Baines in Arch Dis Child Educ Pract Ed 2011.

Medicine	Pharmacology	Physiological effects	Dosage	Practical implications
Dopamine	D1, D2, β1, β2 agonist	Increases contractility and vascular resistance. At lower doses, dopamine is claimed to be a vasodilator (acting on dopaminergic and then β-receptors), but at higher doses it has a greater effect on vasoconstriction.	Neonates: 5–20 μg/kg/min PICU starting dose: 3–5 μg/kg/min, maximum dose 20 μg/kg/min May have an effect at 1 μg/kg/min in healthy children	Associated with vasoconstriction so requires a long line or central line
Dobutamine	Predominant β1 agonist	Affects contractility without increasing vascular resistance. Dobutamine has a greater action on β-receptors, producing vasodilation, tachycardia and chronotropy.	Neonates: 5–20 μg/kg/min PICU starting dose: 3–5 μg/kg/min, maximum dose 20 μg/kg/min	Can be infused via peripheral line
Epinephrine	α1, α2, β1, β2 agonist	Increases contractility (with increased vascular resistance at higher doses). Theoretically, epinephrine acts more on the β-receptors than on the α-receptors and so should increase BP by increasing cardiac rate and contractility. Dopamine and dobutamine are less potent and have less peak effect than epinephrine or norepinephrine. All may produce tachycardia. Higher doses lead to receptor desensitisation but can be used sometimes.	Neonates: 100–300 ng/kg/min Others: 0.1 titrated up to 1.5 μg/kg	Associated with vasoconstriction so requires a long line or central line
Norepinephrine	α1, α2, β1 agonist	Norepinephrine has a proportionally greater action on the α-receptors and so increases BP by vasoconstriction.	Neonates: 20–100 ng/kg/min initially, up to 1.0 μg/kg/min as base. Others: 20–100 ng/kg/min initially, up to 1.0 μg/kg/min as base. Higher doses lead to receptor desensitisation	Associated with vasoconstriction so requires a long line or central line
Vasopressin	ADH agonist in arterioles	May replace basal vasopressin levels in cases of severe hypotension.	0.018–0.12 units/kg/h May be used as rescue treatment	
Hydrocortisone			In neonates: 2.5 mg/kg 6 hourly Others: 1 mg/kg 6 hourly	Uncertainty about role as rescue or primary treatment
Milrinone etc	PDE III inhibitor		0.5–0.75 μg/kg/min	

Table 1.4: Summary of effects of inotropic agents, doses and physiological effects. *Table reproduced with permission from Which inotrope and when in neonatal and paediatric intensive care? by M.A. Turner and P. Baines in Arch Dis Child Educ Pract Ed 2011.*

1.17 Supplementary work

The following section describe the introduction and background for the various additional work that arose as a part of the main project.

Variation between staff recorded and continuously downloaded invasive blood pressure in extremely preterm infants

Blood pressure is a frequently measured parameter in the neonatal intensive care unit for monitoring the cardiovascular status. This is commonly achieved using invasive arterial lines in extremely preterm newborn infants. Electronic monitoring of invasive BP monitoring reveals the beat-to-beat variability, which is not captured by one off hourly recording of BP by staff.

Clinicians and researchers have often used the staff recorded BP data from nursing charts to determine the need for inotropic support and to investigate the role of BP in observational studies and clinical trials.

The difference between continuously downloaded BP and staff recorded BP has been studied in the adult critical care unit (Wong et al. 2014) but this has not been explored in extremely preterm infants. Our aim was to compare staff recorded BP and continuously measured BP downloaded from the monitoring systems of extremely preterm infants in the neonatal intensive care unit.

Comparison between invasive and non–invasive blood pressure measurements

Blood pressure can be measured using invasive and non–invasive methods. Invasive BP is considered the gold standard method for measuring BP. In reality, it is not possible for all infants to have invasive BP measurements. In instances where clinicians have been unable to insert invasive lines, clinicians will have to rely on non–invasive BP. We know that non–invasive BP measurements tend to overestimate the BP when compared to invasive BP (Troy et al. 2009, Dannevig et al. 2005). The degree to which the non–invasive measurements overestimate BP depends on the type of equipment used to measure BP.

The aim of this study was to estimate the degree of difference between invasive and non–invasive (measured using both upper and lower limbs) BP in extremely preterm infants.

Chapter 2

Methods

2.1 Location for the study

This study was carried out at The Royal London Hospital, part of Barts Health NHS Trust, which is the largest trust in the National Health Service. The hospital is one of the two perinatal centres providing neonatal surgery in the north east London perinatal network. This tertiary level neonatal intensive care unit has 37 cots. Twenty-five are intensive care/ high dependency cots and twelve special care cots. The unit had 802 admissions accounting for 4227 intensive care unit days, 4424 high dependency unit bed days and 4559 special care bed days in 2015/16 finance years based on BAPM 2001 standards. There were 322 (14%) infants admitted to the unit who were born between 23 to 28 weeks gestation in the 5-year period from 2009 to 2013.

2.2 Funding for the study

This study was funded through multiple channels. A part of the funding was raised by parents of the infants cared for in the neonatal intensive care unit of the Royal London Hospital. This funding was administered through Barts Charity (grant reference number 420/2189). The other half of the funding was through the salary of the researcher who did part time clinical work throughout the 2–year period of the study.

2.3 Data monitoring committee

The data monitoring committee (DMC) group was set up before the start of the study to oversee the trial, monitor safety of the participants, monitor compliance and to give guidance for further management of the trial to the sponsors (Calis et al. 2017, Fleming et al. 2017, DAMOCLES 2005). It consisted of Prof. N. Aladangady and Dr. A. Groves, consultant neonatologists and E. Harley, paediatric research nurse. Dr. A. Groves has ex-

pertise in neonatal haemodynamics. The DMC met before the start of the study and were in constant contact with the research team throughout the period of the study. A report was sent to the DMC after recruitment of every 15 babies. The DMC ensured that there were no adverse outcomes in any of the arms and monitored the recruitment of the study. A final report was sent to the DMC at the end of the study after completing recruitment. There were no concerns raised by the DMC during this study and research team was allowed to complete recruitment of the planned sixty babies.

2.4 Ethical approval and parental consent

This study has research ethics committee approval from The National Research Ethics Service (NRES) Committee London —Surrey Borders (REC reference number 12/LO/1553) and the study has been registered with the International Standard Randomised Controlled Trial Number (ISRCTN 83507686). The full study protocol can be accessed in section 7.2.

When the delivery of an extremely preterm infant was anticipated, the parents were approached by a member of the research team. Verbal and written information (section 7.3) was given to the parents explaining the details of the study. The parents were given sufficient time to consider the information given to them and were approached again at a later point. Where parents have agreed to participate in the study before the birth of the baby a signed assent was obtained. Formal written informed consent was obtained once the baby was born. For unexpected and precipitate deliveries, parents were approached, given verbal and written information and consented after delivery. For babies who were transferred ex utero, telephone consent was obtained initially (with the nursing looking after the baby confirming this with the parents and signing the consent as a witness) followed by written consent when the parents visited the neonatal unit. The parent information sheet was in

English and where parents spoke languages other than English, an health advocate was used to aid in explaining the study and obtaining consent. Parents were given a copy of the signed consent form (section 7.4) for their documentation. All parents were offered the option of withdrawing from the study if they changed their mind but were informed that data collected up to that point would be used for analysis.

The person with parental responsibility was approached for obtaining informed parental consent. If the parents were married at the time of birth of the baby, either the father or mother could give consent. If parents were unmarried, the mother was approached for consent. This practice is in keeping with the current legislation requirements in the United Kingdom (Legislation.gov.uk 1989).

Prior to the start of the study, we carried out a survey among parents of extremely preterm infants in our neonatal unit who agreed that further research in this area is required. Among the parents surveyed, 7/10 (70%) reported that they will let their infants participate if such a study were to be carried out in the neonatal unit.

After completing recruitment and analysis of the data, a detailed summary of the findings from this study was submitted to the research ethics committee.

2.5 Participants of the study

Babies were eligible to take part in the study if they were:

i. Born $\geq 23^{+0}$ weeks and $\leq 28^{+6}$ weeks gestation

ii. Admitted to the neonatal intensive care unit

iii. Not known to have major congenital malformations

iv. Recruited and randomised in the first 12 hours of life

2.6 Study design

This study was carried out between February 2013, and April 2015 in a single tertiary level neonatal intensive care unit. It was a 3–arm, open label randomised controlled trial. Eligible babies were enrolled and randomly assigned to one of the following three arms (interventions):

i. **Active:** If the baby's MABP falls below 30 mmHg for more than 15 consecutive minutes.

ii. **Moderate:** If the baby's MABP falls below the baby's gestational age in mmHg (commonest UK criteria) for more than 15 consecutive minutes.

iii. **Permissive:** Blood pressure will only be supported if there is clinical evidence of impaired tissue perfusion, or MABP falls below 19 mmHg for more than 15 consecutive minutes.

2.6.1 Inotropic support

Infants were managed as per the neonatal unit's cardiovascular guideline prior to randomisation. Once the infants were enrolled, depending on which arm they were randomised to, the inotropic support was either commenced or weaned as per the written guideline available at the cot side of every infant participating in the study. This ensured that both the medical and nursing staff looking after the infant were aware of the intervention and inotropic support requirements of infant.

Commencing inotropic support

Once a baby was recruited into the study, depending on what arm they were randomised to, blood pressure was supported using inotropes as per the unit's established cardiovascular guideline, which was available at the cot side of every baby participating in the study (section 7.5). The clinician looking after the baby decided upon the choice and dose of the inotrope to be commenced. After measuring the physiological parameters, the researcher informed clinicians if the baby's cardiac output was less than 150 ml/kg/min or if the common carotid blood flow was less than 2 SD than what was expected.

Weaning of inotropic support

Babies received inotropic support until the blood pressure stabilised above the intervention level. Once the blood pressure has stabilised above the intervention level, inotropic support was adjusted as per a formal written guideline which was available at the cot side for all babies participating in the study (section 7.6). For babies who are transferred ex utero on inotropic support, once recruited and depending on what arm they are randomised into, the inotropic support was adjusted to maintain blood pressure for the particular arm that they are randomised to.

In order to achieve consistency, a pragmatic approach was adopted for weaning the inotropic support of infants recruited to the study regardless of the arm that they were randomised to. This written guideline which was the unit policy was as follows:

i. If MABP >10 mmHg above the intervention level for 15 minutes, wean inotropic support immediately by at least 20% and continue to wean 1-2 hourly whilst MABP stays at this level.

ii. If MABP >5 mmHg above the intervention level, begin to wean after 6 hours of stability at this level and continue to wean 2-4 hourly whilst MABP stays at this level.

iii. If MABP is 1-5 mmHg above the intervention level, begin to wean after 12 hours of stability and continue to wean 2-4 hourly whilst the MABP stays at this level.

The actual blood pressure threshold levels were clearly documented in the guidance for weaning of inotropic support for all infants across various gestational ages in the study. An example of the blood pressure weaning regimen for all three arms of the study can be found in section 7.6.

2.7 Outcomes of the study

2.7.1 Primary outcomes

i. Mean arterial blood pressure during the first week of life (collected hourly for the first 12 hours and 4-hrly thereafter)

ii. Use of inotropes and duration of their use

2.7.2 Secondary outcomes

i. Death or parenchymal brain abnormality on cerebral ultrasound before discharge home

ii. Death before discharge home from hospital

iii. Periventricular leucomalacia (on cranial ultrasound at or before 36 weeks corrected gestational age)

iv. Parenchymal periventricular haemorrhage (on cranial ultrasound on Day 1 and by one week)

v. Other periventricular haemorrhage

vi. Acquired gastrointestinal pathology (necrotising enterocolitis, perforation or GI surgery)

vii. Treatment for patent ductus arteriosus (drugs or ligation)

viii. Maximum serum creatinine in the first 2 weeks of life

ix. Maximum serum potassium level

x. Duration of respiratory support

xi. Chronic lung disease (defined as oxygen dependency at 36 weeks post-conceptual age)

xii. Use of postnatal steroids including hydrocortisone

xiii. Duration of neonatal care at each BAPM care level (this is the basis for charging for care and a good marker of health service costs)

xiv. Neurodevelopmental status at routine developmental follow-up

2.8 Randomisation and masking

Randomisation for this study was achieved using stratification in individual weeks of gestation with permuted blocks of 3 patients. We used a random number generator using Microsoft® to allocate the blocks. The allocation was concealed in sequentially numbered opaque sealed envelope which was performed by Dr. S. Kempley. After the infant was recruited and consented, another member of the team (Dr. S. Pereira) who was not involved in the allocation process, opened the allocated envelope and informed the clinical team to which arm the infant was recruited to. A written information sheet was given to the clinical team clearly specifying the blood pressure at which clinicians looking after

the infant should intervene. The clinicians were blinded to block size and all allocations were concealed and administered in the correct order with no deviations in study protocol.

2.9 Sample size

For the primary outcome measure of inotrope usage, previous data showed rates of 15% where permissive hypotension was practised Dempsey et al (Dempsey et al. 2009)) and 68% in our unit using active management (unpublished data). In order to carry out this pilot study, sample size was calculated on the basis of a 15% vs 65% difference in proportions, requiring 18 patients in each group for 80% power at 5% significance level. The 15% vs 65% difference in proportions for inotrope use were different from a clinicians point of view. A higher proportion of inotrope use would entail more intensive care resources and personnel. As this study is not powered to detect the differences in clinical outcomes, this difference in the proportion of inotropic support would not not make much difference from a patient point of view.

We also carried out power calculation for a three-level, one-way ANOVA on the parametric secondary outcome of common carotid artery flow. Our previous study (Sinha et al. 2006) on common carotid artery flow showed a mean of 20 ml/kg/min in stable preterm infants with a standard deviation of 4.9 ml/kg/min. It would require 20 patients to demonstrate a difference of 5ml/kg/min with 80% power at 5% significance level (Minitab® v16.1.0). We therefore aimed for 20 infants to each arm (a total of 60 infants).

2.10 Data collection and study measurements

The demographic data for the infants participating in the study was obtained from the notes and the national electronic neonatal database Badger Net. The relevant pregnancy and delivery details were obtained from the maternal notes. Gestational age was determined by fetal ultrasound scan (dating scan) performed before 14 weeks in 59/60 (98%) infants and in one infant at 26 weeks (infant was born at 26^{+5}). The blood and other relevant biochemistry results were obtained from the hospital electronic patient records. Once infants were discharged to their local hospitals, follow up data was obtained through the national neonatal database Badger Net. The long term neurodevelopmental follow up details will be obtained form formal neurodevelopmental assessments performed at 2 years of age.

Blood pressure, clinical outcome measures and treatment instituted will be recorded onto standard case report forms that has been designed for this study. Although invasive blood pressure measurement is considered the gold standard method, in clinical practice, it is not unusual to have blocked or malpositioned arterial lines. Therefore not all babies can have invasive blood pressure monitoring. In light of this and to avoid an unacceptable drop out in recruitment, we have decided to include babies who did not have invasively measured blood pressure as well. Babies with invasive blood pressure monitoring will be analysed separately.

Before measurement of the various physiological variables, care was taken to ensure that measurements were timed before cares, babies were minimally handled before performing the scan and the water based ultrasound gel sachets were left in the incubator to warm up before use. All babies enrolled in the study had initial echocardiography to rule out obvious congenital cardiac anomalies.

To determine whether the observed effects were primarily due to blood pressure or blood flow differences, detailed physiological studies were performed in all the babies in the study using echocardiography. The following physiological variables were measured:

i. Right common carotid artery blood flow

ii. Left ventricular output and assessment of ductus arteriosus shunt

iii. Superior mesenteric artery blood flow velocity

iv. Amplitude integrated EEG

v. Downloaded continuous blood pressure data from monitors

A full set of measurements were performed on day 1 (6-24 hours) and day 3 (48-96 hours). Additional cardiovascular measurements were performed in selected patients at other pertinent times, such as when extremes of blood pressure or treatment are encountered.

In our neonatal unit we use General Electric (GE) Healthcare (Carescape B850 monitors) for the purposes of monitoring of vital signs. Before the start of the study, care was taken to ensure that the date and time across all recording equipment's were synchronized with the date and time on the GE monitoring systems. If there was a discrepancy, the time on the laptop and aEEG machines were changed to match the GE monitoring systems. The GE monitoring systems used in the neonatal intensive care unit are serviced and calibrated on a yearly basis by the clinical engineering department based at The Royal London Hospital.

We used Philips iE33 (Philips Medical Systems Bothell WA) ultrasound machine for all our Doppler measurements. The ultrasound scanner is serviced and undergoes calibration checks by the clinical engineering department on an annual basis. This ultrasound machine

is equipped with 3 types of transducers, all of which were utilised to measure the various physiological parameters in the study. The ultrasound probes use piezo-electric crystals to generate sound waves. The most commonly used synthetic piezo-electric ceramic in medical imaging is PZT (lead-zirconate-titanate). The 3 types of ultrasound probes that were utilized for this study are described below.

2.10.1 Measurement of carotid blood flow

Figure 2.1: Figure and schematic showing the 7-15 MHz linear array transducer used to measure the right common carotid artery blood flow.

A 7 -15 MHz (L15-7io, Philips iE33 Medical systems, Bothwell, WA) linear array transducer probe was used for this measurement. This 7 to 15 MHz probe has piezo-electric crystals, which are arranged in parallel. This probe weighs 50 grams and has a footprint of 1 cm by 3.2 cms. The transducer face is flat and it produces images, which are rectangular in shape. The sound waves produced are parallel to each other. This high frequency probe produces images with high resolution and is excellent in imaging structures very close to the body surface typically superficial blood vessels.

The probe was placed longitudinally on the right side of the neck to visualize the right common carotid artery. Colour Doppler was used to highlight the right common carotid artery. After correcting the angle of insonation and optimizing sample volume size to include the entire width of the artery at the site of sampling, pulsed wave Doppler was used to acquire waveforms. Intensity weighted mean (IWM) velocities were automatically ac-

quired by the ultrasound machine using a minimum of six arterial waveforms (Figure 2.2). The probe was then rotated through 90 degrees to visualise the cross section of the right common carotid artery. Using a two dimensional cross sectional view, the image was optimised to visualise two hyperechoic regions directly opposite to each other. This represents the end on view of the vessel and ensures the ultrasound beam is travelling perpendicular to the cross section of the artery. The measurement was done from the trailing to leading edge of both the hyperechoic area signifying the inner borders of the intima of the artery and diameter (d) measured (Figure 2.3). A total of 5 maximum systolic diameters were measured and the average calculated to improve accuracy. The carotid blood flow was then calculated using the formula (IWM) * π ($d^2/4$) * 60. This would provide the carotid blood flow in millilitres per minute. This value was then divided by the birth weight to obtain the right common carotid blood flow in ml/kg/min.

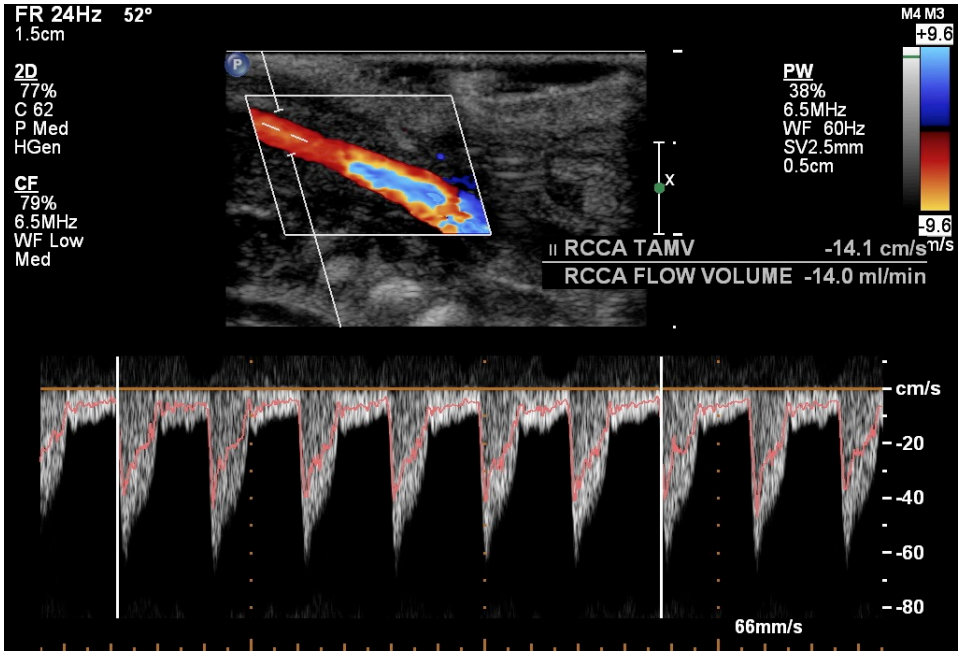


Figure 2.2: Longitudinal view of the right common carotid artery with the Doppler sampling gate placed in the artery. The time averaged mean velocity was calculated from 6 artefact free waveforms.

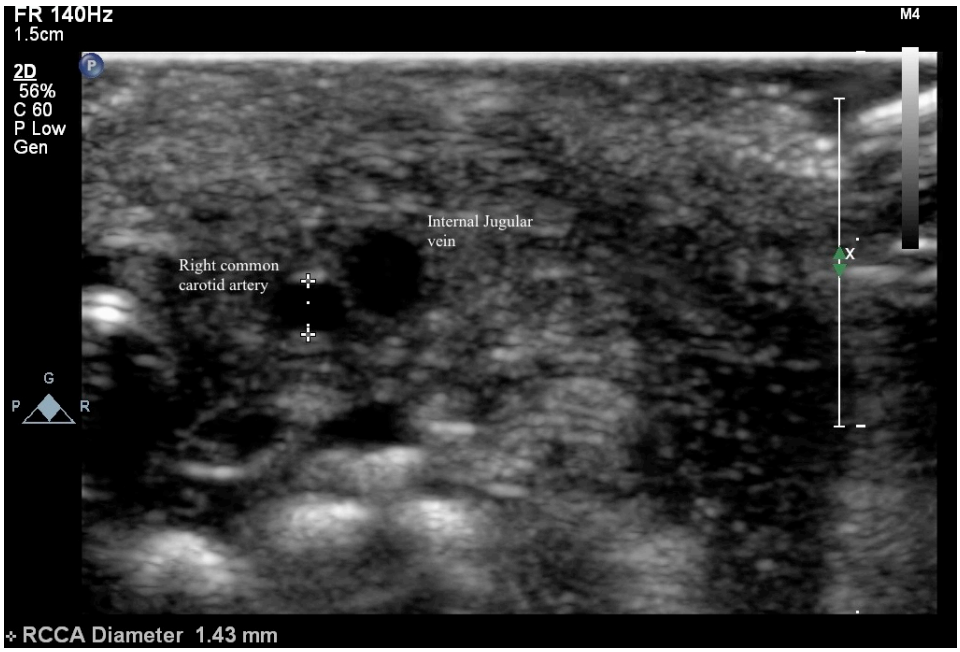


Figure 2.3: Cross section of the right common carotid artery along with the internal jugular vein. Diameter measurements are taken from the trailing to leading edge of the opposing echogenic area that represents the intima of the artery.

The common carotid artery was used to measure carotid blood flow using well established techniques (Sinha et al. 2006). The right common carotid artery was used, as it was furthest away from the patent ductus arteriosus and therefore less likely to be influenced by the patent ductus arteriosus (PDA).

The origins of the right and left common carotid arteries differ. Whilst the left common carotid artery is directly a branch of the arch of the aorta, the right common carotid artery arises from the brachiocephalic artery behind the sternoclavicular joint. The right common carotid artery therefore only has a cervical part unlike the left common carotid artery that has a cervical and thoracic part. The carotid arteries have a similar course on both sides. In the lower part of the neck, the arteries are separated by a small gap containing the trachea. Above this level, each common carotid artery is contained within the carotid sheath of the deep cervical fascia. Within the carotid sheath, the internal jugular vein lies lateral to the artery and the vagus nerve lying between the two structures. The carotid

triangle, which is part of the anterior triangle of the neck, containing the common carotid artery and its branches; the external and internal carotid artery is limited posteriorly by the sternocleidomastoid, anterioinferiorly by the omohyoid muscle and superiorly by the stylohyoid and posterior belly of the digastric muscle. At the level of the 3rd to 4th cervical vertebrae, it divides into the external and internal carotid arteries. In roughly 12% of cases, the right common carotid arises above the sternoclavicular joint or it may even arise directly from the aortic arch. The left common carotid artery varies in origin more than the right common carotid artery (Stranding 2005).

The brain being a highly vascular organ demands about 15%-20% of the cardiac output and utilizes 20% of the total oxygen consumption of the body (Kandel et al. 2012). The brain receives its blood supply from the 2 internal carotid arteries and the 2 vertebral arteries that form a complex anastomosis, the circle of Willis, at the base of the brain. The branches of the common carotid artery; external carotid artery has multiple branches that supply the thyroid gland, tongue, face and scalp. The internal carotid artery supplies most of the ipsilateral cerebral hemisphere, eye, nose and forehead. After entering the cranial cavity, the internal carotid artery terminates as the anterior and middle cerebral arteries. The vertebral arteries, which are branches of the subclavian artery, and its branches supply the upper spinal cord, the brain stem, cerebellum and the occipital lobe of the cerebrum. The blood flow through the adult brain is approximately 1000 millilitres per minute. The blood flow rate through each carotid artery is approximately 350 millilitres per minute (Kandel et al. 2012). It can be concluded that the 2 internal carotid arteries contribute about 70% of the cerebral blood flow and the vertebral arteries contribute the remaining 30%.

2.10.2 Measurement of superior mesenteric artery blood flow

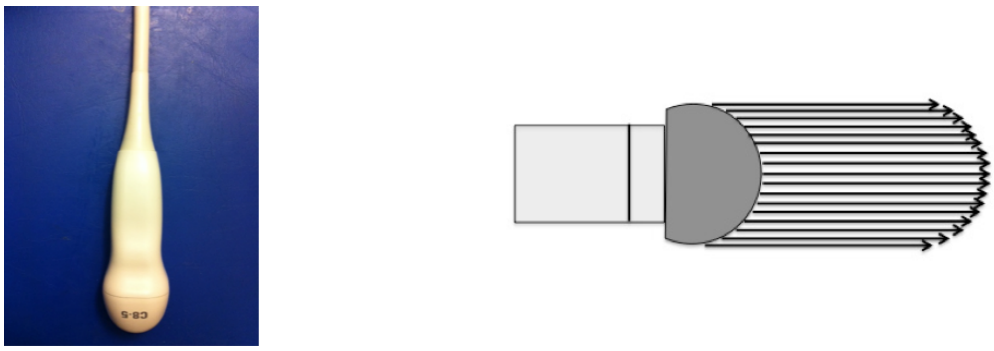


Figure 2.4: Figure and schematic showing the 5-8 MHz curvilinear transducer used to measure the superior mesenteric artery blood flow.

A 5-8 MHz (C8-5, Philips iE33 Medical systems, Bothwell, WA) curvilinear transducer was used for this measurement. This probe has piezo-electric crystals, which are arranged in parallel or in concentric rings. It weighs 82 grams and has a footprint of 0.7 cm by 2.7 cm. The transducer face is curved and the probe produces images, which are sector or pie-shaped. This curvilinear probe uses lower frequency which helps in deeper penetration and therefore useful in visualising intra-abdominal structures.

The probe is placed just below the xiphisternum and angled to the left to obtain a longitudinal view of the abdominal aorta. After adjusting the probe and optimising the image the superior mesenteric artery was visualised arising distal to the coeliac artery. Colour Doppler was used to highlight the superior mesenteric artery. After correcting the angle of insonation, pulsed wave Doppler signal was sampled from the superior mesenteric artery from away from its junction with the abdominal aorta (Figure 2.5). A minimum of six arterial waveforms was measured to calculate the mean of the peak systolic velocity, peak systolic velocity, end diastolic velocity and pulsatility index. This method of estimation of superior mesenteric artery blood flow was found to have good intra and inter rater reproducibility. The intra observer variation was acceptable with 95% of the repeated

measurements being expected to fall between 20% to 35% of the first measurement for lower and higher velocities respectively (Kempley 2011, Fang et al. 2001). The intraclass correlation for different measurements obtained between observers varied from 0.72–0.83 (Weir et al. 1995).

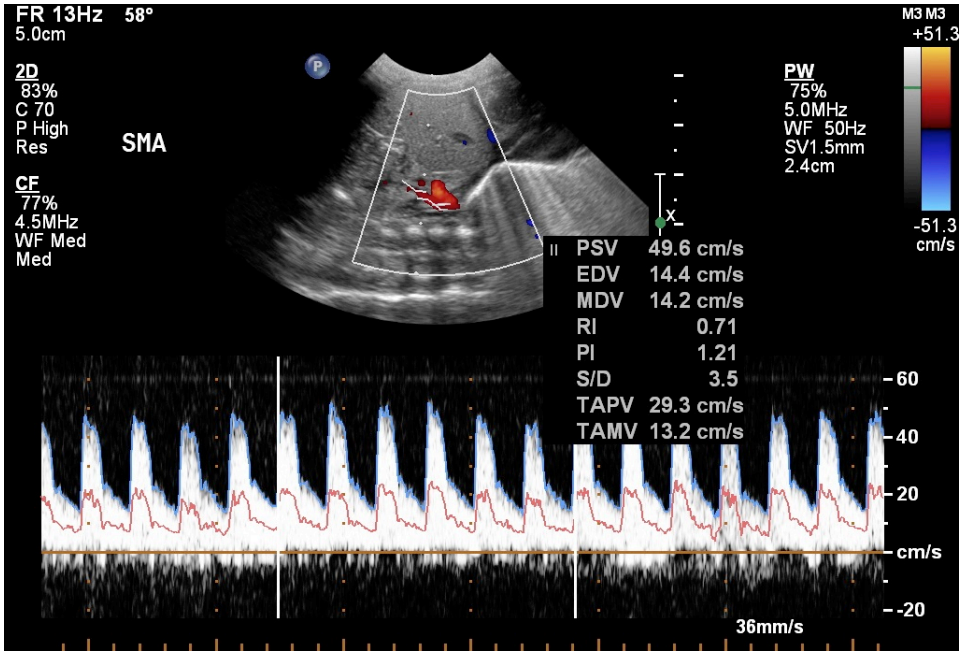


Figure 2.5: Longitudinal view of the superior mesenteric artery with the Doppler sampling gate placed in the artery. The time averaged mean velocity was calculated from 6 artefact free waveforms.

2.10.3 Measurement of the cardiac output

Figure 2.6: Figure and schematic showing the 4-12 MHz sector array transducer used to measure the cardiac output.

A 4-12 MHz (S12-4, Philips iE33 Medical systems, Bothwell, WA) sector array transducer was used to measure the cardiac output. This probe has piezo-electric crystals, which are arranged as a parallel array or an annular array. This probe weighs 52 grams and has a footprint of 1 cm by 1.4 cm. This sector or phased array transducer has a small footprint, but produces a large depth of field. It produces a fan shaped image, which is narrow close to the transducer but widens out away form the transducer. This principle is useful in visualising deeper structures for example in the chest where the probe can be placed in between the ribs and the ultrasound waves are beamed in between the ribs.

The long axis parasternal view was used to visualize the cross sectional diameter of the aortic valve. The aortic diameter was measured at the hinge points of the aortic valve at the end of systole. An average of 5 recordings were used to calculate the diameter (D) (Figure 2.7). From the apical 5-chamber view, angle corrected pulse wave Doppler measurements were obtained from the left ventricular outlet. The pulse wave Doppler gate was placed at the level of the aortic valve. Five arterial waveforms were used to manually trace the velocity time integral (VTI) and heart rate (HR) (Figure 2.8). The cardiac output was then calculated using the formula HR * π ($D^2/4$) * VTI which derived the cardiac output in millilitres per minute. This value was then divided by the birth weight to obtain the cardiac output in ml/kg/min.

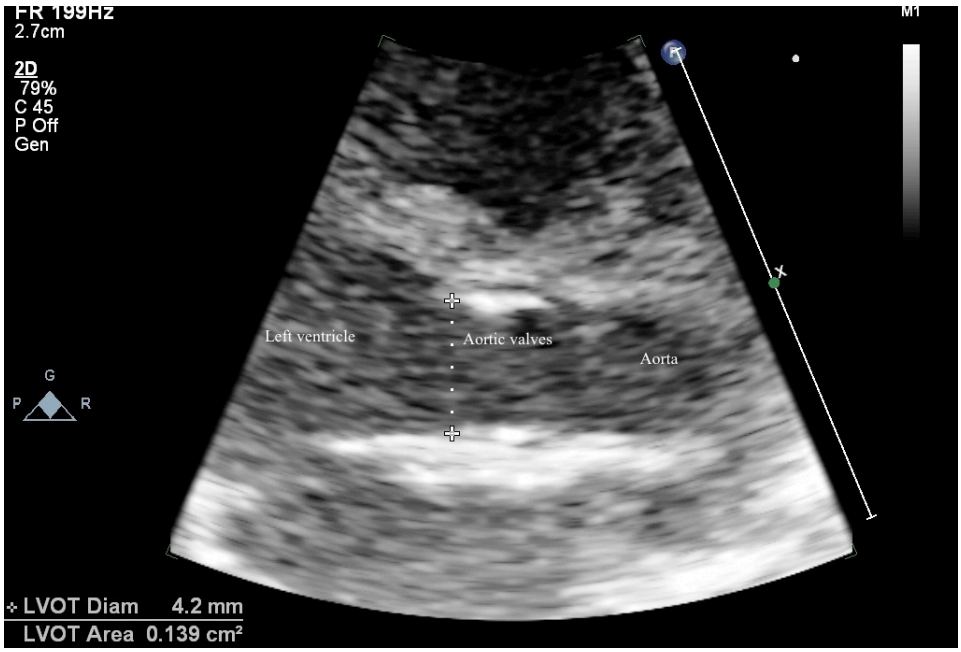


Figure 2.7: Cross-sectional view of the left ventricular out flow tract. Using the long axis parasternal view, the aortic valve diameter measurements are taken from hinges of the aortic valve.

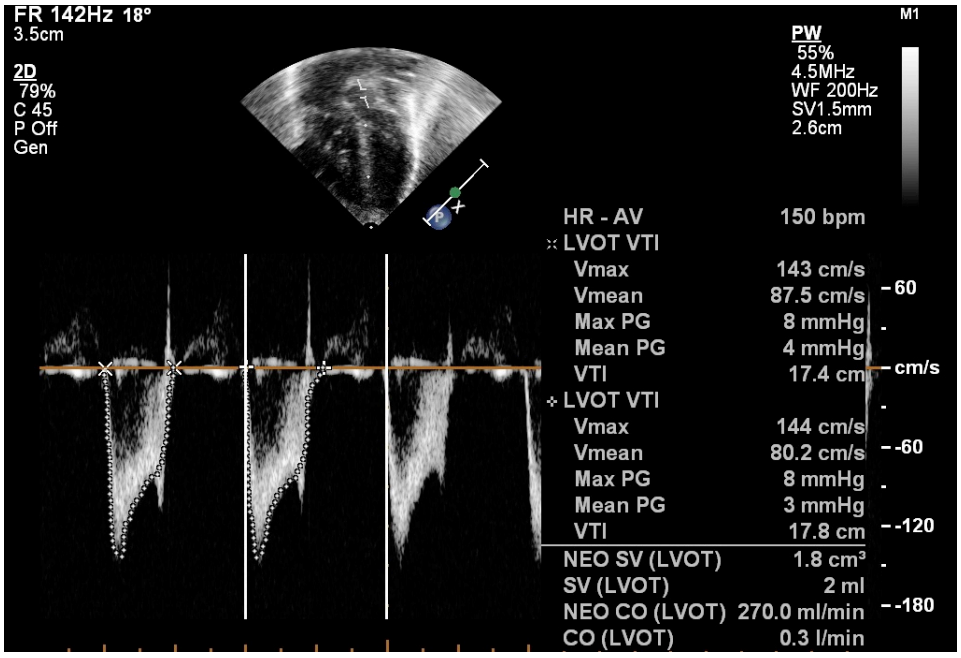


Figure 2.8: Figure showing the cardiac output being measured from the apical 5-chamber view. The sampling gate is placed in the left out flow tract distal to the aortic valve.

Cardiac output in this study refers to the left ventricular output expressed as ml/kg/min. It can be argued that early on in life, left ventricular output will be influenced by the presence of fetal channels (Evans and Iyer 1994). Hence some critics may argue that the right

ventricular output should be used for estimation of cardiac output. However, this too is not without its limitations. The right ventricular output can also be affected by atrial shunting. Hence, in the absence of an ideal method, we have chosen to use the left ventricular output as a marker of cardiac output.

PDA was assessed using the parasternal short axis view. The view of the main pulmonary artery and the confluent branch pulmonary arteries were obtained. Once this view was obtained, the image was then optimised by narrowing the sector width to improve image quality, adjusting the focus to the area of interest, adjusting the Nyquist level so that the colour signal filled the width of the ductus arteriosus. The sampling gate then was placed on the ductus arteriosus. After angle correction, the maximum velocity was estimated using pulse wave Doppler if velocity was less than 2 m/sec or continuous wave Doppler used if the maximum velocity was more than 2 m/sec (Figure 2.9). The size of the PDA was measured using both 2D and colour Doppler views at the narrowest point along the course of the PDA (Figure 2.10).

2.10.4 Measurement of invasive arterial blood pressure

Invasive blood pressure measurements were monitored using a 3.5 French size umbilical arterial catheters that were inserted as part of standard neonatal care soon after the baby was admitted to the neonatal intensive care unit. The tip of the umbilical arterial catheter was ideally positioned in thoracic aorta between the 6^{th} and 10^{th} thoracic vertebrae. The umbilical arterial lines were continuously infused with 0.5 millilitres per hour of heparinised saline to keep them patent. The umbilical arterial catheter, once connected to the transducer was calibrated by holding the transducer at the level of the heart in the mid axillary line. Calibration is done by closing the 3-way valve to the baby and opening

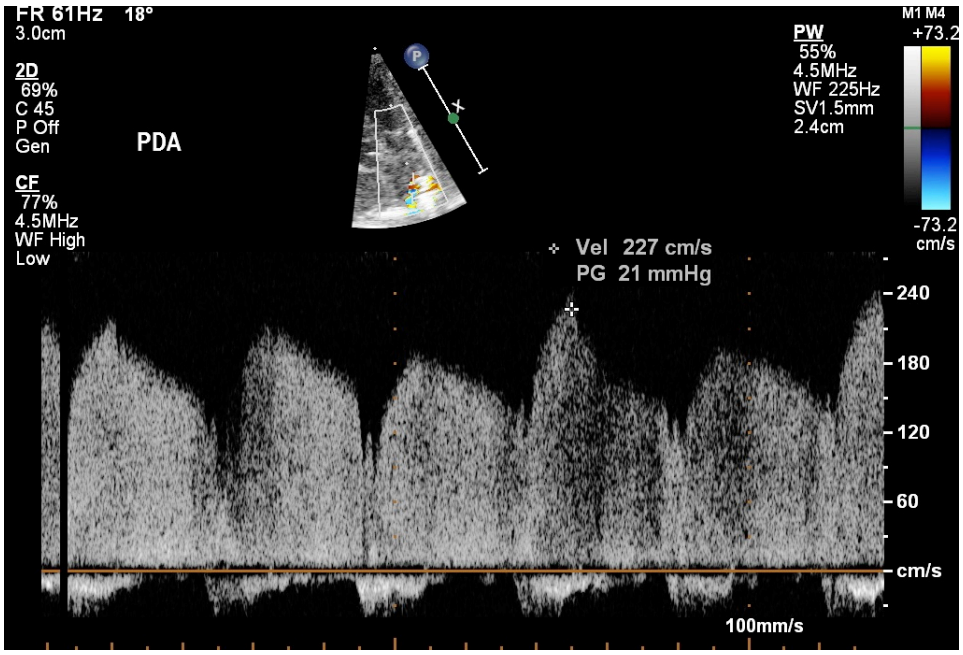


Figure 2.9: Continuous wave Doppler used to measure the blood flow velocity through the patent ductus arteriosus. The marker represents the peak velocity of flow in the PDA.

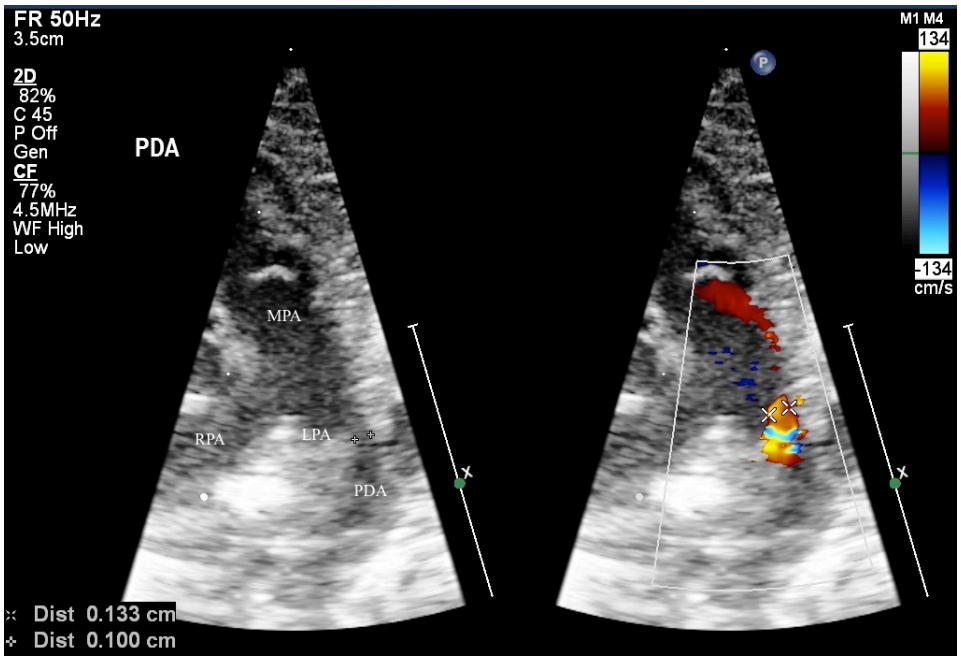


Figure 2.10: Figure showing the two-dimensional and colour Doppler view of the patent ductus arteriosus obtained from the short axis view. MPA –main pulmonary artery, RPA –right pulmonary artery, LPA –left pulmonary artery, PDA –patent ductus arteriosus.

it to air and pressing the 'zero' button on the GE monitor. After calibration, the 3-way valve was opened to the baby. The line being infused with heparinized saline was checked thoroughly to ensure that it was devoid of air bubbles that could potentially dampen the arterial signal and therefore give a falsely low systolic and high diastolic blood pressure.

The heparinized saline was changed every 24 hours and the line was re-calibrated every time as described above. The umbilical arterial catheter was only used if it sampled well and that there was a good trace of arterial waveforms on the monitor. In instances where the umbilical arterial catheters were malpositioned or blocked, a peripheral arterial line was inserted and patency was maintained with heparinised saline. After calibrating the line and ensuring the above precautions were taken, the peripheral arterial line was used for sampling and monitoring blood pressure. We use GE Healthcare medical systems (Carescape Monitor B850), which traces the heart rate, systolic, diastolic and mean blood pressure and the respiratory rate.

2.10.5 Measurement of non-invasive arterial blood pressure

The commonly used oscillometric technique was used to measure the non-invasive blood pressure for infants participating in this study. This was achieved using GE Healthcare medical systems (Carescape Monitor B850) in our neonatal intensive care unit. Non-invasive blood pressure was measured using an appropriate sized cuff that covered three-fourth of the length of the limb that was being measured. Prior to the measurements, staff looking after the infant ensured that the baby was settled, was not handled or undergone any painful procedures prior to measurement of the blood pressure. Measurements were done every 15 minutes if the blood pressure was low and less frequently (once every 4 hours) once the blood pressure stabilised above the threshold level that the baby was randomised to. If unexpectedly high or low blood pressures were obtained, staff re-measured it. (pre–specified blood pressure values were not agreed upon but re-measurements were carried out if there was a difference of 15–20 mmHg). The decision to insert umbilical arterial lines to monitor invasive blood pressure or to measure non-invasive blood pressure measurements was entirely up to the discretion of the clinician looking after the baby. The gestation of the infant and the level of respiratory support was taken into consideration to

determine which infants required invasive blood pressure monitoring using umbilical lines and which infants did not.

2.10.6 Recording of amplitude-integrated electroencephalogram (aEEG)

aEEG activity was recorded for 72 hours in the majority of the infants using a 2–channel BRM3 monitor (BrainZ Instruments, Natus Medical Incorporated, Ontario, Canada) which provides a digital raw EEG signal output as well as the aEEG. After preparation of the scalp using NuPrep^{TM} gel (Nuprep, DO Weaver & Co, Aurora, CO, USA) to reduce skin impedance, neonatal hydrogel electrodes (Neonatal Sensors, Natus Medical Incorporated, Ontario, Canada) were placed on the fronto–parietal regions (C3–P3, C4–P4) bilaterally according to the international 10-20 system (Jasper 1957) (Figure 2.12). A 2–hour artifact and seizure free electroencephalogram trace, confirmed by two researchers, recorded before and after measurement of the carotid artery blood flow was chosen for analysis. A 2–hour long epoch was chosen to capture all periods of electrical quiescence which may be missed if very short epochs were chosen. Cross cerebral aEEG (P3–P4) was analyzed using Research Analyze software (BrainZ Instruments, NZ) for median values of minimum and maximum amplitude; measures of lower and upper aEEG margin respectively, percentage of time that the minimum amplitude was below 5 microvolts. Care was taken to ensure that the date and time were synchronized with the date and time on the GE healthcare medical systems and the date and time on the laptop. BRM3 monitors are serviced and calibrated on a yearly basis by the clinical engineering department at The Royal London Hospital.

Calculation of interburst interval/ discontinuity

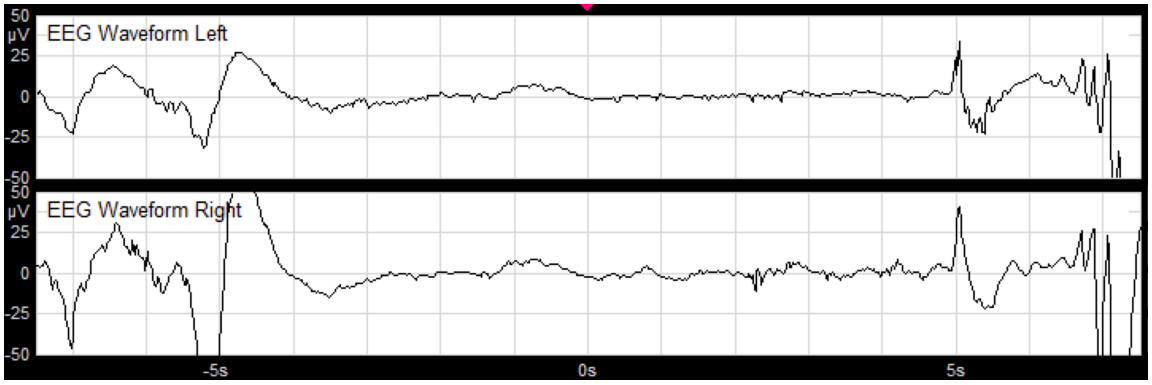


Figure 2.11: Raw electroencephalographic trace from a 24 weeks gestation infant showing a interburst interval.

Single channel cross–cerebral (P3–P4) raw EEG data were exported to Microsoft Excel® and continuity was analysed in 1–minute epochs with software that we developed using MATLAB (The MathWorks, Inc., MA, USA) using a similar approach to that previously described (Wertheim et al. 1991). The system detected an interval if the absolute amplitude of the raw EEG was less than 20 μV with respect to the baseline for at least 6 seconds (Figure 2.11). The 20 μV threshold was chosen to reflect the fact that the EEG from preterm newborns is represented by more high voltage low frequency wave forms in contrast to full term newborns (Werner et al. 1977) and to help reduce any effects of background noise. The threshold level was thus chosen so as to reliably identify EEG bursts and distinguish them from background noise artefact in view of visual assessment of raw EEG characteristics. The 6 seconds criterion for defining an interval was chosen in order to exclude quiescent periods that are normally associated with tracé alternans. P3–P4 raw EEG was analysed, as C3P3 and C4P4 tend to be more susceptible to movement artefact. For each recording the mean of the total interval length per epoch, the discontinuity value, was calculated and expressed in seconds; this can also easily be expressed as a discontinuity proportion since the epoch length is constant.

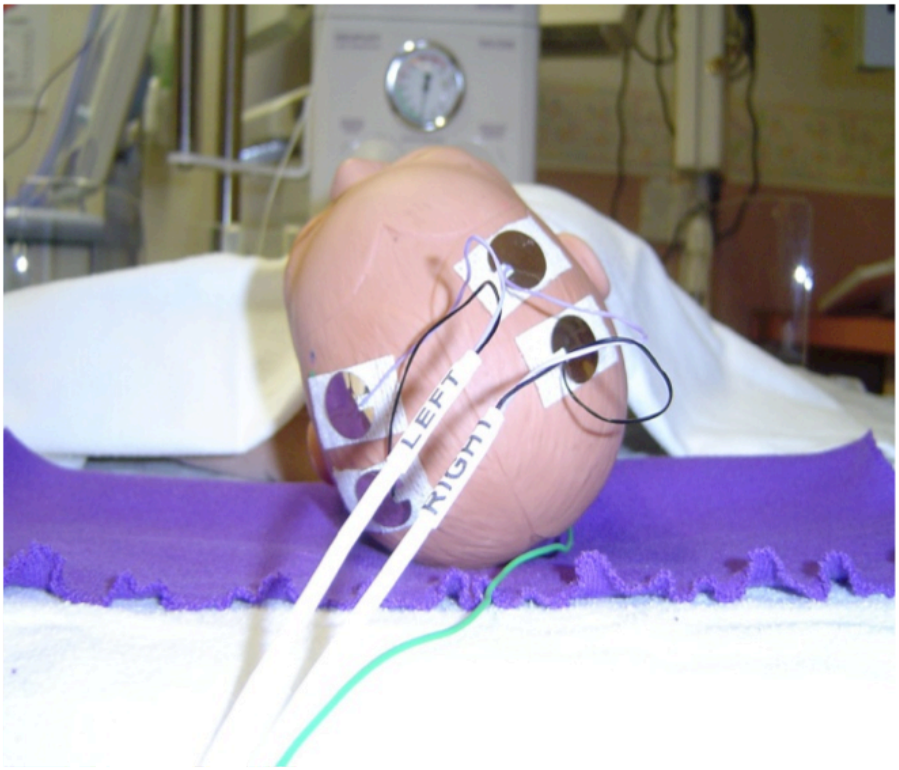


Figure 2.12: Figure demonstrating the ideal positioning of electroencephalographic leads used for the study.

Image courtesy –Dr. Divyen Shah

2.10.7 Blood gas parameters

Arterial blood gas was used for all infants in whom invasive arterial lines were present and capillary blood gas in those who did not have invasive arterial lines. All blood gas parameters used for the study were obtained from single measurements that were recorded as close as possible to the measurement of the physiological parameters.

2.10.8 Markers of peripheral perfusion

Lactate measurements were obtained from single blood gas measurements recorded as close to the measurement of the physiological parameters. Capillary refill time was measured by the researcher at the time of measurement of physiological parameters. Urine output was calculated for the preceding 12 hours from the time of measurement of the physiological parameters. Oliguria was defined as a urine output of less than 1 ml/kg/hour.

2.11 Classification of cerebral pathology on cranial ultrasound scan

All CrUSS in this study were performed using the standard views. The neonatal unit policy was to perform scans on day 1, day 3, day 7, day 14, day 28 of postnatal age and then at 36 weeks gestation or prior to discharge home. All CrUSS performed in the first week, and the CrUSS performed as close as possible to 36 weeks gestation, were reviewed independently by two neonatal consultants who were blinded to the allocation. Periventricular haemorrhage (Figure 2.13) was classified on the Papile scale (Papile et al. 1978), with documentation of late findings of porencephalic cyst, periventricular leukomalacia and ventricular dilatation (ventricular index > 97th centile) (Levene and Starte 1981, Shim et al. 2012).

The initial blinded agreement on the findings were reported and in case of disagreement, consensus between the two consultants were arrived at with minor adjustments.

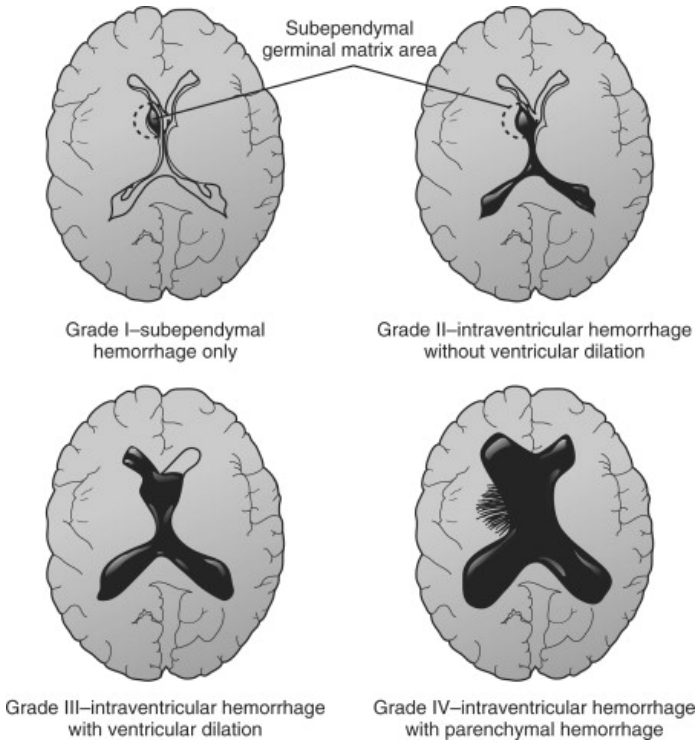


Figure 2.13: Schematic illustrating the various grades of periventricular haemorrhage. *Image reproduced with permission from Intraventricular Hemorrhage and Posthemorrhagic Hydrocephalus by V.C. Smith in Primary Care of the Permature Infant 2008.*

2.12 Supplementary work

The methods used for the various supplementary work done is described in this section. This work was carried out on the same cohort of infants who were recruited to the randomised controlled trial between February 2013 to April 2015. The main exclusion criterion for the supplementary work was the presence of major congenital malformations.

2.12.1 Variation between staff recorded and continuously downloaded invasive blood pressure in extremely preterm infants

Blood pressure measurement

All infants who only had invasive blood pressure monitoring were selected for this work. Invasive blood pressure measurements were acquired using methods described earlier (section 2.10.4). As a part of the blood pressure trial, staff recorded the blood pressure on an hourly basis for the first 12 hours of life and then every 4 hours for the first week of

life in the blood pressure chart used for the study. Mean blood pressure was downloaded every 10 seconds for the first week of life onto a laptop for the majority of the babies. The mean blood pressure recordings downloaded every 10 seconds was averaged for an hour (360 data points) and this was compared with the hourly staff recorded blood pressure.

Statistical analysis

Normally distributed data was described using mean (standard deviation) and non Gaussian distribution was described using median (interquartile range). Correlation between variables was measured using Spearman's rho correlation. Bland–Altman plot was used to asses the agreement between the two methods of measurement.

2.12.2 Comparison between invasive and non–invasive blood pressure measurements

Participants

We compared paired invasive and non–invasive blood pressure measurements obtained from randomly selected infants admitted to the neonatal intensive care unit of the Royal London Hospital in September 2015. This cohort of infants was different from the main BP study cohort. Only infants who had invasive arterial lines were selected so that comparison could be made with non–invasive blood pressure.

Blood pressure measurements

Non–invasive blood pressure measurements were done every 15 minutes for 6 readings using the upper and lower limbs using previously described methods and equipment (section 2.10.5). Paired measurements were defined as a non–invasive blood pressure and invasive blood pressure measurement obtained within 5 minutes of each other. The readings obtained were compared with the invasive blood pressure measurements. Invasive

blood pressure measurements were recorded first followed by non–invasive blood pressure obtained from both limbs. Staff were free to choose the limb but generally avoided the one where an intravenous access was present. The limb used for measurement of the blood pressure was recorded. Care was taken to ensure that the appropriate size cuff which covered three-fourth of the limb was used and the infant was not handled or had painful procedures before the measurements.

Statistical analysis

Data was summarised using mean (SD). Analysis, limits of agreement and 95% CI were examined using Bland–Altman plot.

2.13 Statistical methods

Data was tested for normality of distribution using the Kolmogorov-Smirnov test and the normality plot. Continuous data was summarized using mean (SD), or median (IQR) for skewed distributions. Blood pressure was represented as median (IQR) and as mean for the purposes of illustration. Where appropriate, log normal data was used for analysis of skewed data.

For comparison of means between repeated measurements among subjects, the paired samples t test was used and the non–parametric equivalent for non–normal distributions. Comparison of independent means between subjects was performed using independent samples t test. Correlation between normally distributed variables were performed using Pearson's correlation and Spearman's rho was used for skewed data. The correlation coefficient, r gives a measure of the strength of association between the two variables being tested. However, the sample size plays an important role here and could influence the

strength of association in that a large sample size can give a statistically significant result even with a small correlation coefficient. Labelling systems exist to grade the strength of association, with correlation coefficient of 0.36–0.67 indicating moderate correlations and > 0.68 indicating high correlations (Taylor 1990). A more meaningful way to interpret statistical testing is to use the coefficient of determination, r^2. The coefficient of determination, r^2 is the percent of variation in the value of the dependent variable that is explained by variations in the value of the independent variable (Taylor 1990)

To reduce multiple pairwise testing, data was always examined initially for effects across the three treatment arms, using tests for ordered effects on outcomes (assuming Active > Moderate > Permissive). For normally distributed continuous variables this was performed using ANOVA and predefined contrasts, for non-normal data the Jonckheere-Terpstra test was used. For categorical outcomes the Chi-squared test with Linear-by-Linear association was used; Fishers exact test was used when predicted cell numbers were <5, or for non-ordered effects. Analyses were performed using SPSS v22 (Chicago, IL, USA). Differences were considered to be statistically significant at $p < 0.05$.

As an exploratory pilot trial, tables give statistical significance without correction for multiple testing. The most commonly used method for correction of multiple testing is the Bonferroni correction which controls the familywise error rate. This is achieved by dividing the familywise error rate (which is usually 0.05) by the number of tests. The resulting value would be the new threshold for tests to be considered significant. The Bonferroni correction is usually helpful for a small number of multiple comparisons where one or two variables may be expected to be significant. However, when large number of multiple comparisons are performed, this method is not ideal as it results in a high rate of false

negatives. This is overcome by using the Benjamini-Hochberg (Benjamini and Hochberg 1995, McDonald 2014) procedure of correction. This method involves controlling the false discovery rate, which is the proportion of significant results that are actually false positives. This is done by ranking the individual p-values from smallest to largest. Each p-value is then compared to its Bejamini-Hochberg critical value, $(i/m)Q$, where i is the rank, m is the total number of tests and Q is the false discovery rate. The largest p-value that has a $p<(i/m)/Q$ is significant and all p-values smaller than it are also significant. For analysis in this work, we used a false discovery rate of 5% for each analysis.

Statistical analysis for validity work was performed using Bland–Altman plot (Bland and Altman 1986) with limits of agreement. Reliability between raters, within raters and test–retest reliability was analysed using Intraclass correlation (ICC) (Shrout and Fleiss 1979, Landers 2015), absolute agreement, using two–way mixed ANOVA where raters were fixed with random measurements.

2.14 Data storage

All hard copies of data collection forms used for the study had anonymised data which was identifiable only through study numbers. The data collection forms along with other documents of the study were stored in the site file. This was stored in a secure filing cabinet in the consultant's office at the neonatal unit of the Royal London Hospital which had restricted entry with a number lock. All electronic data was stored in a password protected desktop computer at the Centre for Genomics and Child Health, Blizard Institute, Queen Mary University of London and a backup copy was stored in the university's secure electronic storage device system. All the patient clinical notes were archived in the hospital medical records department where it will be stored for a period of 20 years.

2.15 Validity and reliability

It is important for researchers and clinicians to know whether a measurement performed using a particular equipment is precise and consistent. Validity and reliability work was performed on equipments that was used to measure the various physiological variables in this study. Non–invasive blood pressure was measured using GE Healthcare medical systems (Carescape Monitor B850) and the measurement of other physiological variables were performed using Doppler ultrasound.

Validation work studies using live subjects can be challenging for several reasons; variance between subjects, lack of data on what is 'normal', excessive handling immediately after birth is not well tolerated and performing multiple examination on one subject raises ethical issues. Hence, a flow phantom model was constructed with input from the medical physics department using data collected from extremely preterm infants over a period of time.

In order to maintain continuity in the flow of presentation, for this section on validity and reliability, I have included the introduction, methods, results and discussion of this work in the following pages.

2.15.1 Validity of non–invasive blood pressure monitoring systems

Introduction

There are various non–invasive blood pressure measurements systems available commercially. Our medical systems were calibrated using equipment from the medical physics department of the Royal London Hospital. The non–invasive blood pressure measuring systems was checked for precision using FLUKE BP Pump 2 (Fluke Biomedical Corp, Carson City, NV, USA) simulator before the start of the study to evaluate how precise these were against the gold standard at different ranges of blood pressure measurements that could be encountered in infants born at this gestational age.

Methods

Seven randomly chosen monitoring units at the neonatal intensive care unit of the Royal London Hospital were selected. The simulator was dialled, by a member from the medical physics department, to deliver mean blood pressures of 22 mmHg, 40 mmHg, 60 mmHg and 80 mmHg as these were the range of blood pressure that would be potentially encountered in the study. The GE blood pressure cuff took three readings at each of these 4 blood pressure levels settings. The average of the mean blood pressure at each blood pressure level was calculated. Statistical analysis of these measurements were carried out using Spearman's correlation coefficient and Bland–Altman plot.

Results

A total of 28 paired measurements were performed. The mean (SD) simulator and cuff mean BP was 50.5 (22.1) mmHg and 50.7 (21.5) mmHg respectively. The mean (SD) difference between cuff and simulator mean BP was 0.179 (0.79) mmHg. The cuff mean BP and simulator mean BP measurement values are shown in table 2.1. There was good correlation between the cuff mean BP measurements and the simulator measurements,

r=0.971, p<0.001 (Figure 2.14). Bland–Altman plot showed the mean (SD, 95% CI) difference between the two methods to be 0.179 (0.79, -0.1 to 0.5) mmHg with lower LoA (95%CI) of -1.38 (-1.88 to -0.87) mmHg and upper LoA (95% CI) of 1.73 (1.22 to 2.24) (Figure 2.15).

Sl No	Simulator mean BP (mmHg)	Cuff mean BP (mmHg)	Difference [simulator - cuff] mean BP (mmHg)	Average (simulator and cuff) mean BP (mmHg)
1	22	23.6	-1.6	22.8
2	40	39.6	0.4	39.8
3	60	60	0	60
4	80	79.6	0.4	79.8
5	22	23.3	-1.3	22.65
6	40	39.6	0.4	39.8
7	60	60	0	60
8	80	80.3	-0.3	80.15
9	22	23	-1	22.5
10	40	40	0	40
11	60	59.3	0.7	59.65
12	80	79.6	0.4	79.8
13	22	24	-2	23
14	40	40.3	-0.3	40.15
15	60	59.3	0.7	59.65
16	80	79.3	0.7	79.65
17	22	23	-1	22.5
18	40	40	0	40
19	60	59	1	59.5
20	80	79	1	79.5
21	22	23.3	-1.3	22.65
22	40	40	0	40
23	60	59.6	0.4	59.8
24	80	80	0	80
25	22	23	-1	22.5
26	40	40.6	-0.6	40.3
27	60	60	0	60
28	80	80	0	80

Table 2.1: Measurements of simulator and cuff mean blood pressure along with the difference and average between them.

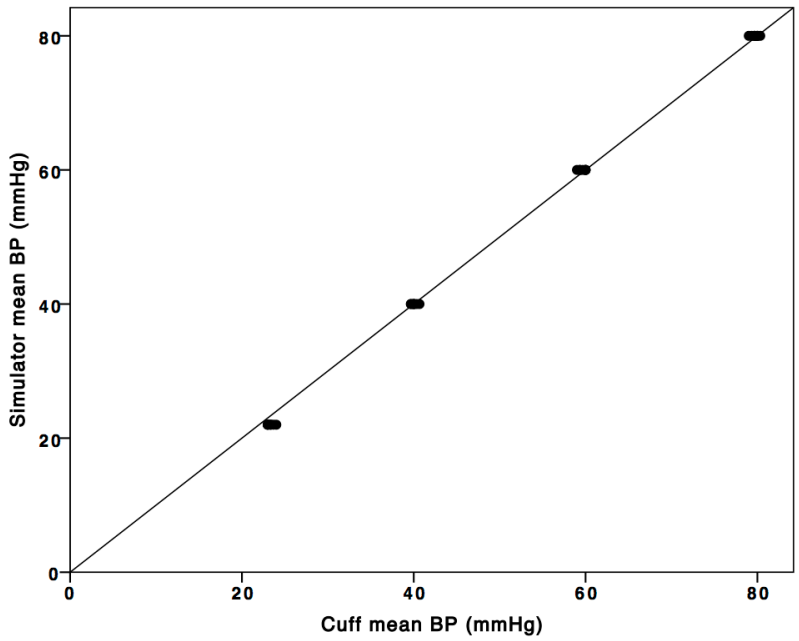


Figure 2.14: Correlation between cuff mean BP and simulator mean BP measurements along with line of equality.

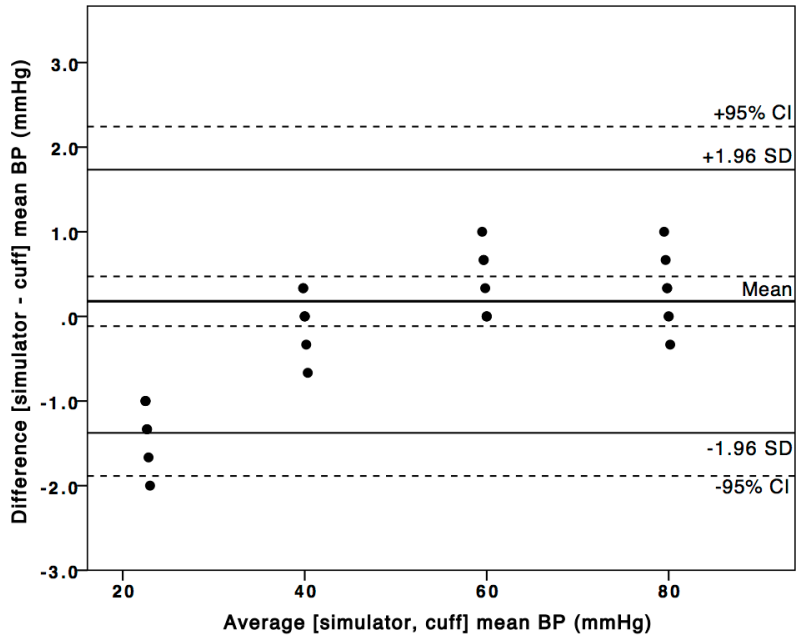


Figure 2.15: Bland–Altman plot between the cuff and simulator mean BP measurements. Solid line represents mean and LoA. Dashed lines represent the 95% confidence intervals.

Discussion

There was very good correlation between the simulator mean BP and cuff mean BP measurement as shown in the scatter plot. However, the true agreement between the two measurements is shown in the Bland–Altman plot. At lower blood pressure measurements, the cuff measurements overestimated whereas at the higher end of blood pressure readings, the cuff measurements were under estimating with 95% CI being -1.9 to 2.2 mmHg. The difference in the lower limit were beyond the lower 95% confidence intervals. There was a trend for the cuff measurements to over-estimate at lower blood pressures by approximately 2 mmHg and under-estimate by approximately 1 mmHg at higher blood pressure ranges. This work was comparable to findings from work using live subjects which concluded that non–invasive blood pressure tend to overestimate blood pressure (Troy et al. 2009, Dannevig et al. 2005). Under controlled conditions, these differences do not seem to be significant but in a clinical setting these readings may be much more exaggerated due to lots of other factors including movement artefacts and handling during procedures and cares.

2.15.2 Flow phantom model

Phantom production

The phantom model was created in the medical physics department of the Royal London Hospital by Dr. J. Reeves PhD, clinical scientist and Dr. M. Birch PhD, clinical director of the medical physics department. Data were collected by Dr. S. Kempley and Dr. S. Pereira from randomly selected extremely preterm newborn infants who were admitted to the neonatal intensive care unit over a period of time which was used for designing the phantom. After production of the initial phantom, further work examining the validity and reproducibility of the model was carried out with the help of Dr. A. Sinha and medical students; Komal Verma, Stephanie Sakthi Finton–Jones and Robert Krug who were

involved in this work as part of their B.Sc project.

The phantom model consists of three 3–chamber blocks containing tissue mimicking material. Embedded within these chambers were PTFE (polytetrafluoroethylene or Teflon) tubing which had a wall thickness of 0.3 mm. Based on the physiological diameters measured in the extremely preterm infants, three different tube diameters were chosen for the model; these were 0.158 cm, 0.196 cm and 0.244 cm respectively. These tubes were inserted in the chambers at a depth of 1cm from the surface. Each chamber had the vessel inclined at 10, 15 and 20 degrees respectively. This combination of different tube diameters and angle of inclination produced nine chambers (A1, A2, A3, B1, B2, B3, C1, C2, C3). One of the 3–chamber blocks is shown in Figure 2.16.

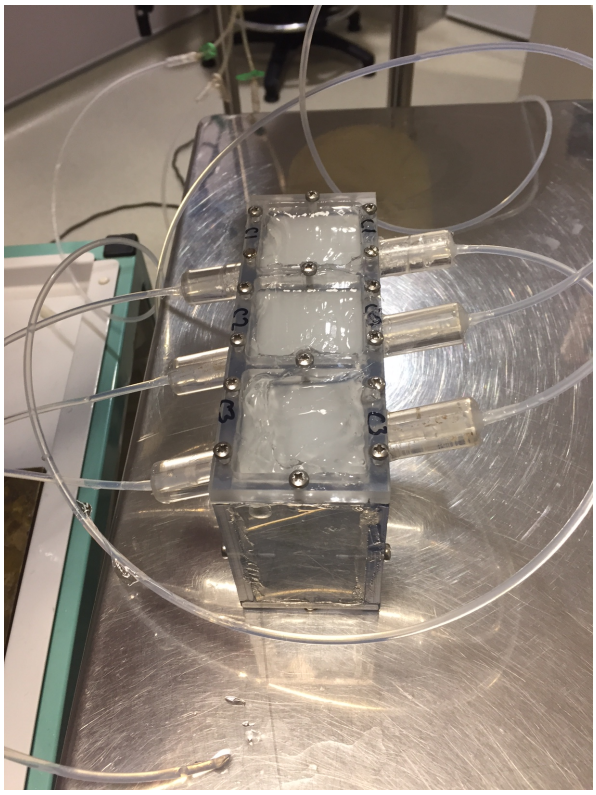


Figure 2.16: Three chamber phantom model

The model was assembled in the neonatal intensive care unit and all measurements were

carried out in the simulation room of the neonatal unit (Figure 2.17). A bag consisting of blood mimicking fluid which was suspended at a height, which ensured a gradient. Blood mimicking fluid flowed out into the chambers at a rate which was controlled using the height of the bag and the flow restrictor. The outgoing fluid having flown through the model was collected onto a container that was placed on a sensitive scale. This scale was then connected to a laptop computer via an analogue to digital convertor which was used to accurately estimate the amount of fluid and thus the flow volume.

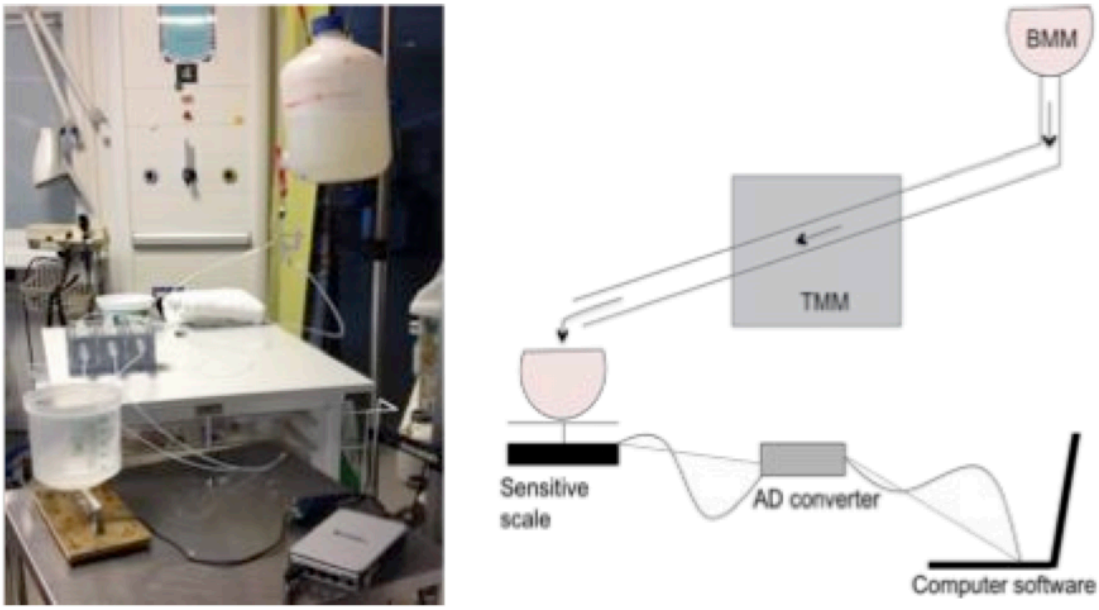


Figure 2.17: Assembled flow phantom model with schematic representation of the model. Arrows shows the direction of flow of fluid. BMM–blood mimicking material, TMM–Tissue mimicking material, AD –analogue–to –digital converter.

Image courtesy –Komal Verma, BSc

Phantom measurements

Each of the nine chambers (A1, A2, A3, B1, B2, B3, C1, C2 and C3) were selected randomly by Dr. J. Reeves and measurements were performed by 2 independent raters (Dr. S. Pereira and Dr. S. Kempley) who were blinded to the characteristics of the model (tube diameters and angle of inclination). To mimic the physiological measurements carried out

in the clinical study, both continuous and pulsatile flow measurements were carried out using the flow phantom. Care was taken initially to ensure that the lines was devoid of air bubbles and the circuit were free from kinks which could influence the velocity and flow. After ensuring these precautions were taken in-between chamber changes and for every measurement, Doppler ultrasound measurements were carried out. When the raters were happy with the quality of the ultrasound signal and pattern of waveform, the image was frozen at which point another member (Dr. J. Reeves) was notified, so that the corresponding time could be noted for later analysis. A third member of the team (medical student) documented the chamber details along with the velocity measurements, measured vessel diameter and angle of insonation ensuring that the other rater was blinded to these measurements.

Continuous flow

Continuous flow in the phantom was achieved by suspending a bag containing blood mimicking fluid at a height with a flow restrictor attached to the end of the line. The resulting gradient ensured that fluid flowed freely from the bag and continuously through the circuit, thus mimicking physiological continuous flow through a blood vessel. The velocity of flow was varied by adjusting the height of the bag containing the blood mimicking fluid and/ or by adjusting the flow restrictor that was attached to the end of the line. In this experiment, the velocity of the fluid flowing through the circuit was varied by adjusting the bag to a certain set high and low point. The bag was topped up with the fluid collected in the container on the scale to ensure that the pressure head did not substantially reduce with subsequent measurements. The flow volume was then calculated using the formula IWMV * π ($D^2/4$) * 60 which derived the flow volume in millilitres per minute. Each rater carried out diameter and velocity measurements using each of the nine chambers (Figure

2.18) at a high and low velocity setting. The various parameters measured by the two independent raters using continuous flow is shown in the appendix section (table 7.1).

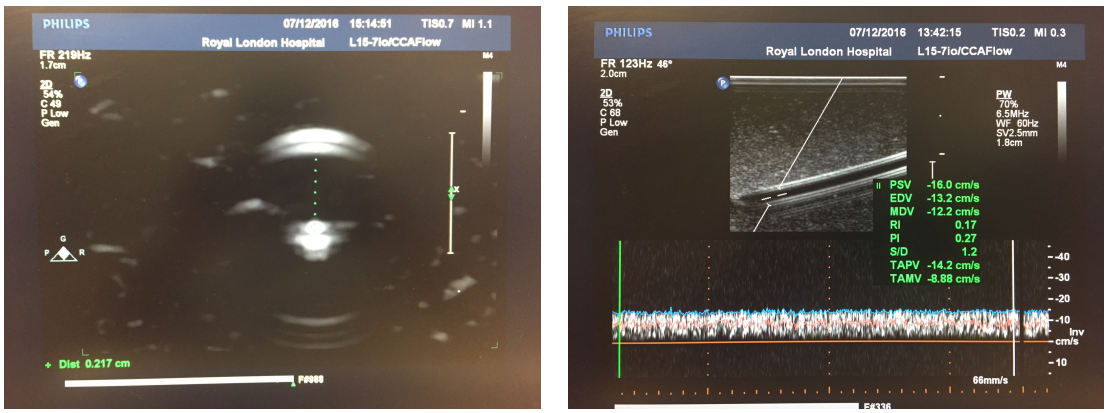


Figure 2.18: Vessel diameter and velocity measurement using continuous flow in the phantom flow model.

Pulsatile flow

The experiment was repeated using pulsatile flow. Pulsatile flow was achieved by including a pump in the circuit (Figure 2.19). This pump was connected to a wheel that consisted of three projections. The tubing forming the circuit was made to run around the three projections of the wheel. When the pump was running, these three projections rotated which compressed the circuit to produce a pulsatile flow that was similar to a physiologically produced pulsatile flow (Figure 2.20). The speed of the pump was adjusted to achieve heart rates between 120 to 150 beats per minute that was comparable to physiological heart rates of infants. As before, two independent raters who were blinded to the model performed repeated measurements sequentially using all three chambers at varying pump speeds. The flow volume (in millilitres per minute) was then calculated using the formula described earlier. The various parameters measured by the two independent raters using pulsatile flow is shown in the appendix section (table 7.2).

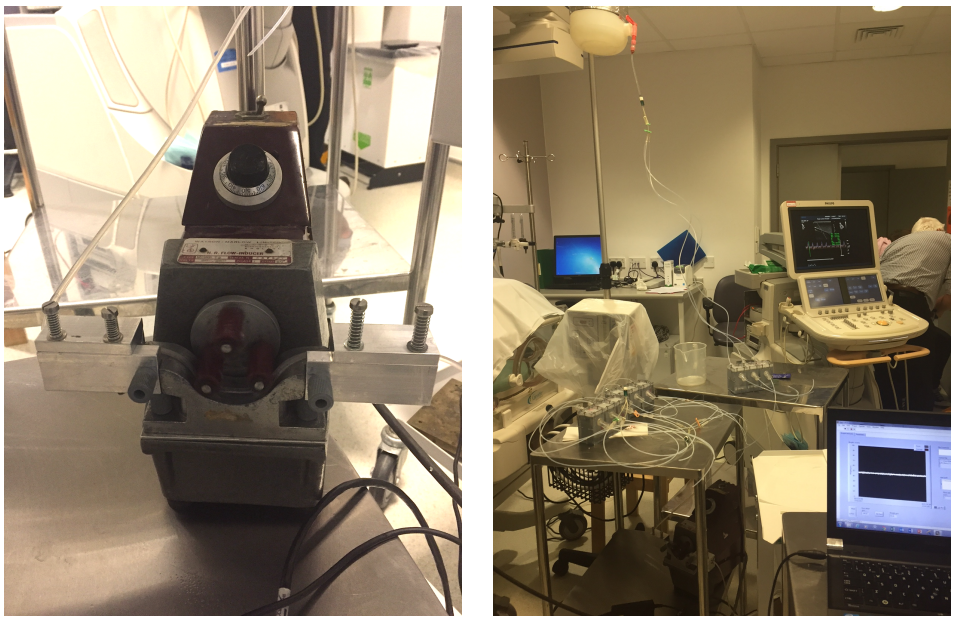


Figure 2.19: Pump used for pulsatile flow was added to the circuit of the phantom flow model.

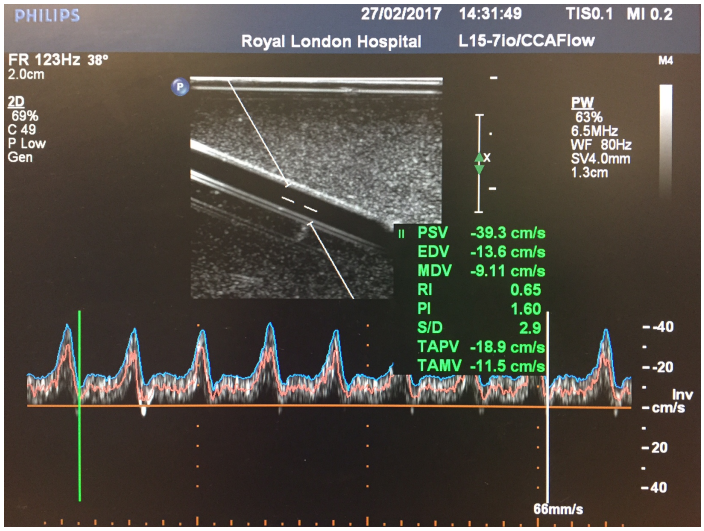


Figure 2.20: Velocity measurement using pulsatile flow in the phantom flow model.

Continuous flow measurements

Multiple measurements were taken alternatively by 2 raters at different speeds. A total of 18 measurements were obtained using all the chambers by each rater (Appendix, table 7.1). Rater 1 (Dr. S. Pereira) and rater 2 (Dr. S. Kempley) were blinded to each others measurements during this experiment. The vessel diameter, flow volume and angle of insonation measurements were compared for both raters.

Measurements were summarised using mean (SD and 95% CI). Correlation between the two raters was visualised graphically. Agreement and validity measurements between the raters was examined using the Bland–Altman plot (Bland and Altman 1986). The reliability between raters and within raters was assessed using intra class correlation (Shrout and Fleiss 1979, Landers 2015). The grading of the strength of correlation (Table 2.2) was assessed using previously described methods (Landis and Koch 1977).

Kappa/ ICC statistic	Strength of agreement/ correlation
< 0.00	Poor
0.00–0.20	Slight
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–1.00	Almost perfect

Table 2.2: Kappa/ ICC statistic and the strength of agreement/ correlation.

Vessel diameter measurements

The mean (SD, 95%CI) diameter of the eighteen measurements for rater 1 and rater 2 were 0.183 (0.032, 0.168 to 0.199) cm and 0.185 (0.032, 0.168 to 0.201) cm respectively. The diameter measurements for all three vessels were underestimated by both raters (Figure 2.21). The Bland–Altman plot (Figure 2.22) for both raters showing that error was far greater for larger diameters. The mean (SD) proportional difference for rater 1 was -7.66 (1.5) % and for rater 2 was -7.26 (1.2) % (Figure 2.23).

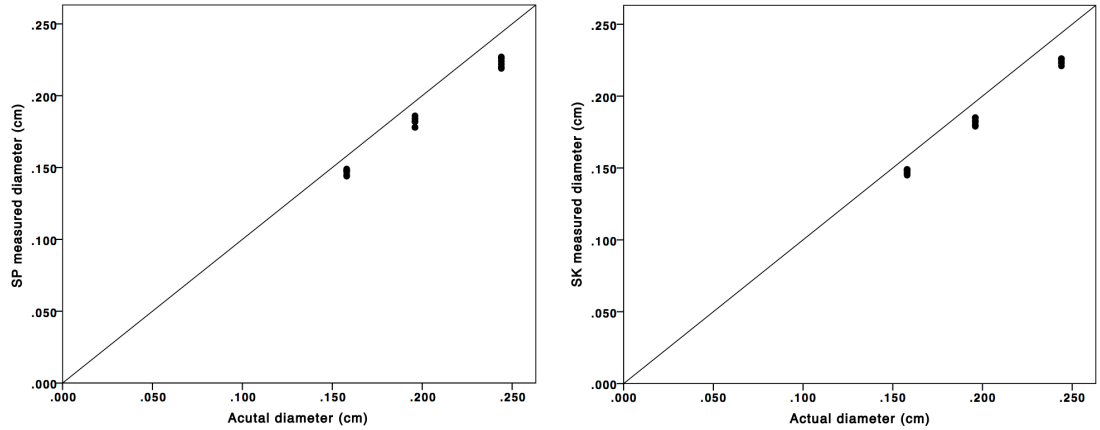


Figure 2.21: Correlation between diameter measured and the actual measurements of the tube along with the line of equality.

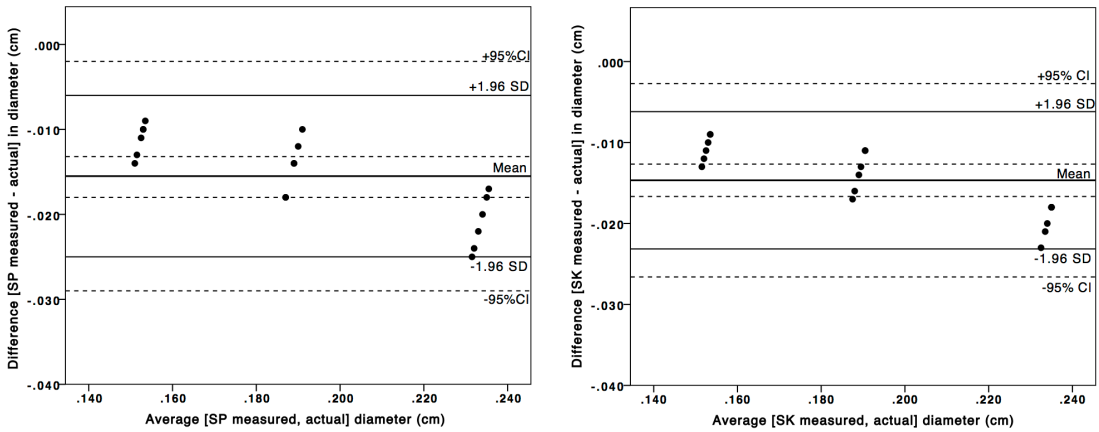


Figure 2.22: Bland–Altman plot of vessel diameter measurements for both raters.

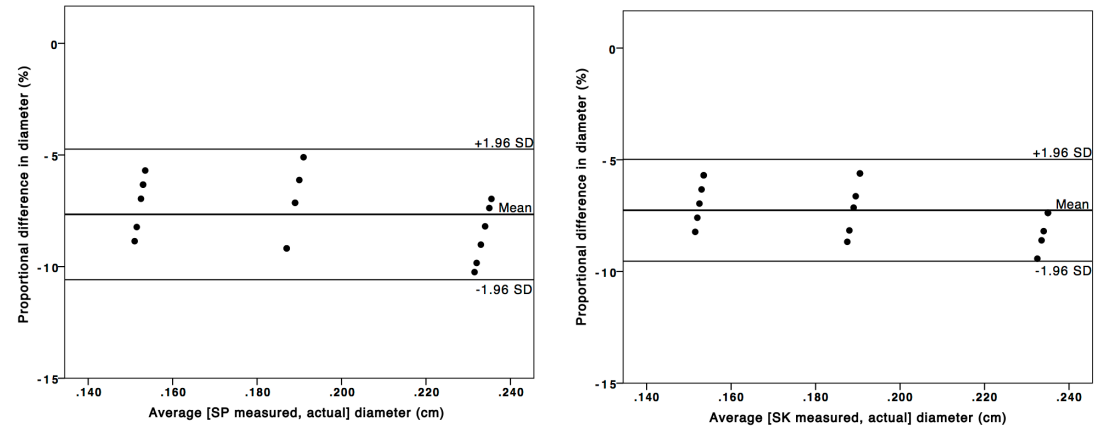


Figure 2.23: Plot illustrating the proportional difference in diameter measurements for both raters.

Continuous flow volume measurements

The mean (SD, 95%CI) flow volumes of the eighteen measurements for rater 1 and rater

2 were 25.2 (14.1, 18.2 to 32.3) ml/min and 26.1 (14.4, 18.9 to 33.3) ml/min respectively. The flow measurements for higher flow volumes were overestimated by both raters when compared to measurements for lower flow volumes (Figure 2.24). The Bland–Altman plot (Figure 2.25) for both raters showing that error was far greater for higher flow volumes. The mean (SD) proportional difference in flow for rater 1 was 7.14 (11.5) % and for rater 2 was 7.64 (11.0) % (Figure 2.26).

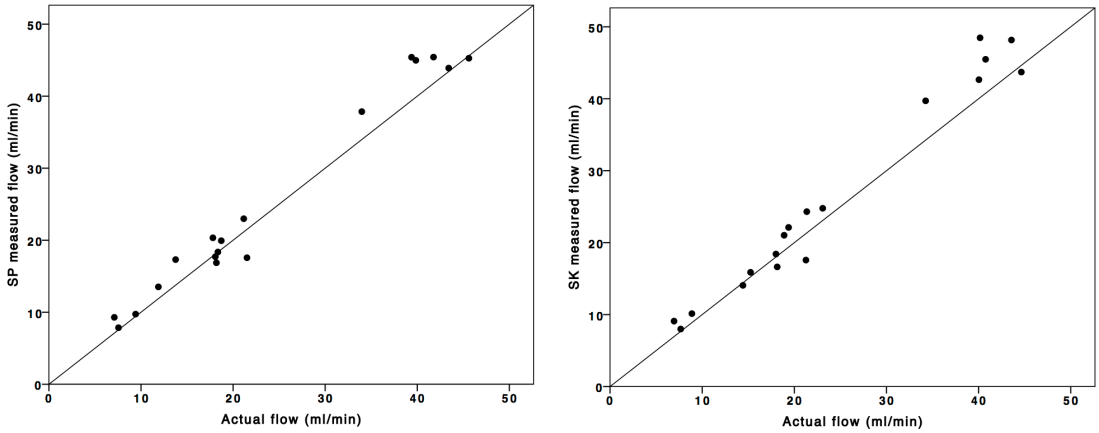


Figure 2.24: Correlation between flow volume measured and the actual measurements along with the line of equality.

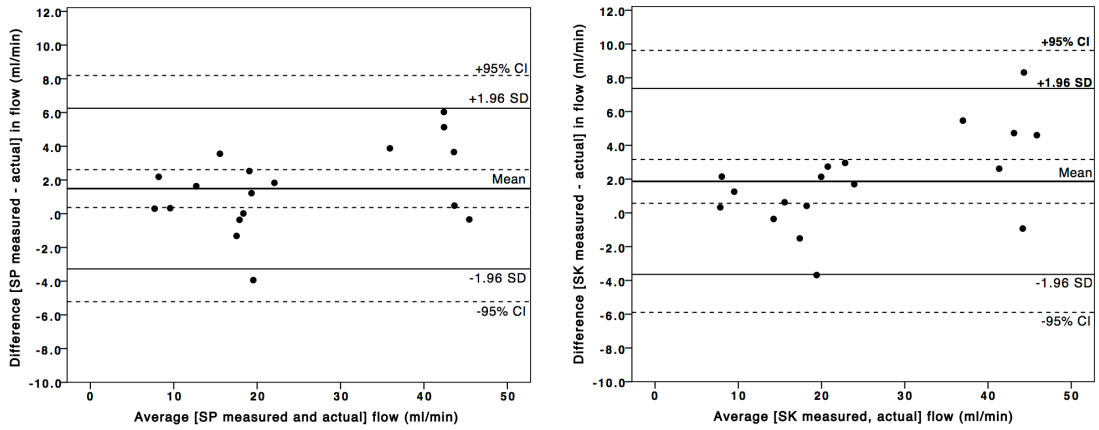


Figure 2.25: Bland–Altman plot of flow volume measurements for both raters.

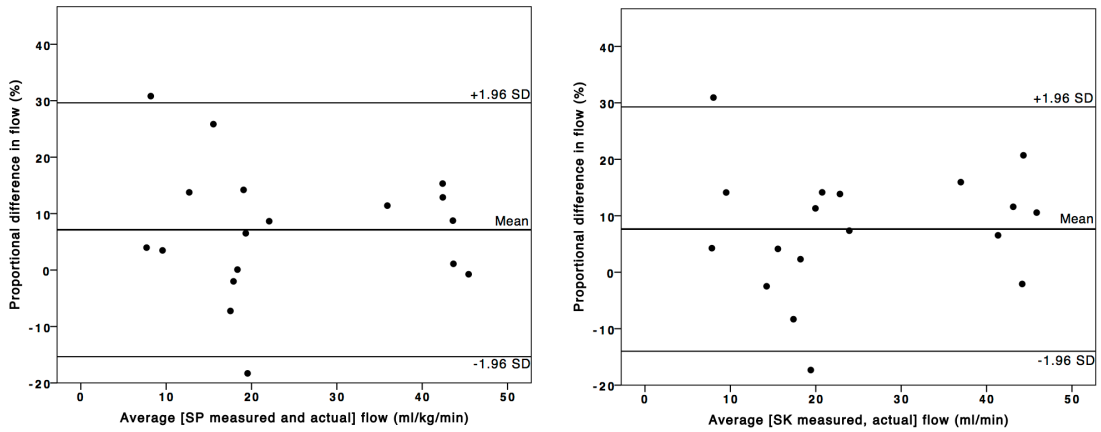


Figure 2.26: Plot illustrating the proportional difference in flow measurements for both raters.

Angle of insonation

The angle of insonation, which plays an important role in estimating the flow volumes, obtained for each of the measurements by the two raters were examined in addition to the diameter and flow measurements. The mean (SD, 95%CI) angle of insonation for rater 1 was 43.7 (4.8, 41.3 to 46.1) degrees and for rater 2 was 42.2 (4.8, 39.8 to 44.6) degrees. The relation between the two raters and difference between the two raters are shown in Figure 2.27.

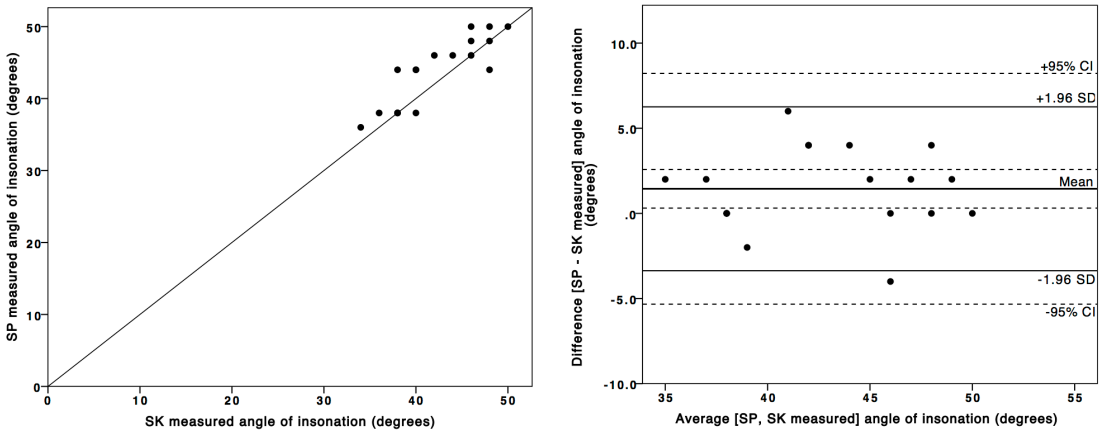


Figure 2.27: Correlation between angle of insonation along with line of equality and Bland–Altman plot between the two raters.

Pulsatile flow measurements

The pulsatile flow measurements were carried out a few months after the continuous flow measurements. This delay was present due to the medical physics department having to modify the flow phantom in order to produce physiological pulsatile flow. Multiple measurements were taken alternatively by 2 raters at different speeds. A total of 24 measurements were obtained using all the different chambers by each rater (Appendix, table 7.2) with some chambers being examined more than twice. Rater 1 (Dr. S. Pereira) and rater 2 (Dr. S. Kempley) were blinded to each others measurements during this experiment. The vessel diameter, flow volume and angle of insonation measurements are compared for both raters.

The observations were summarised using mean (SD and 95% CI). Correlation between the two raters was visualised graphically. Agreement and validity measurements between the raters was examined using the Bland–Altman plot (Bland and Altman 1986). The reliability of measurements between raters and within raters was assessed using intra class correlation (Shrout and Fleiss 1979, Landers 2015). The grading of the strength of correlation (Table 2.2) was assessed using previously described methods (Landis and Koch 1977).

Vessel diameter measurements

The mean (SD, 95%CI) vessel diameter for rater 1 was 0.185 (0.032, 0.172 to 0.199) cm and that for rater 2 was 0.185 (0.032, 0.171 to 0.199) cm. The vessel diameter measurements for all three calibre of vessel were underestimated by both raters (Figure 2.28). The Bland–Altman plot (Figure 2.29) for both raters showing that error was far greater for

larger diameters. The mean (SD) proportional difference for rater 1 was -6.92 (2.4) % and for rater 2 was -6.92 (2.0) % (Figure 2.30).

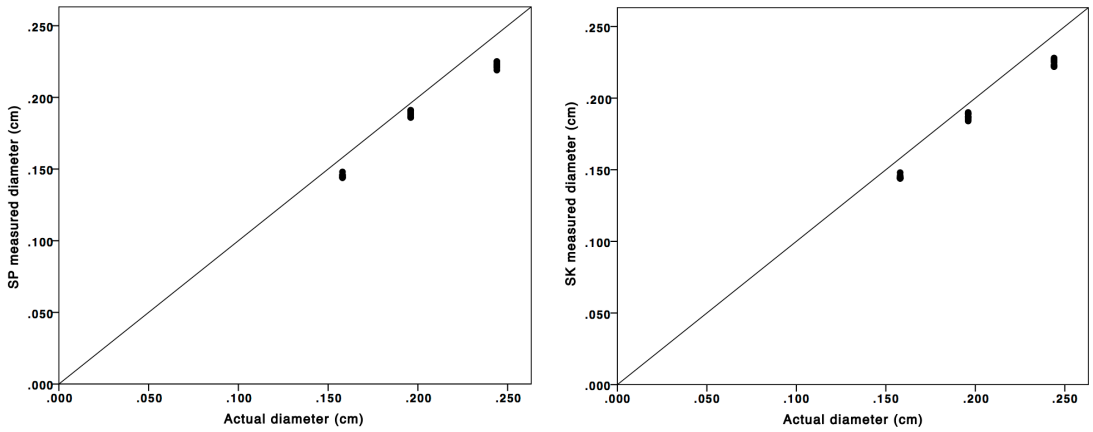


Figure 2.28: Correlation between diameter measured and the actual measurements of the tube along with the line of equality.

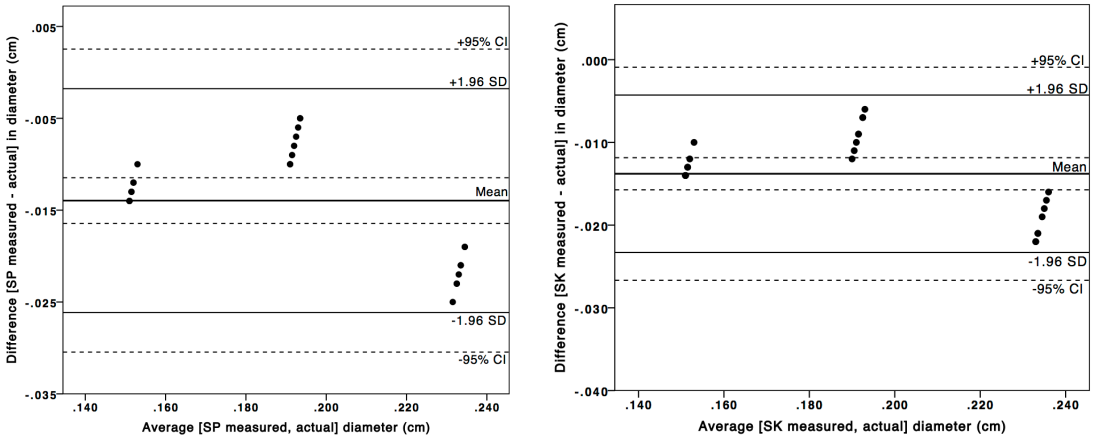


Figure 2.29: Bland–Altman plot of vessel diameter measurements for both raters.

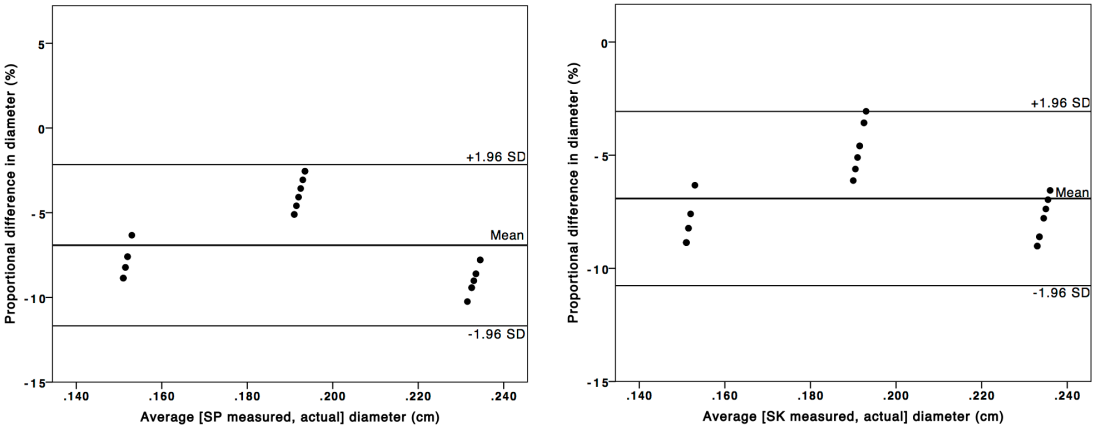


Figure 2.30: Plot illustrating the proportional difference in diameter measurements for both raters.

Pulsatile flow volume measurements

The mean (SD, 95%CI) flow volumes for rater 1 were 24.2 (2.5, 23.1 to 25.3) ml/min and that for rater 2 were 23.1 (2.4, 22.1 to 24.1) ml/min. The majority of flow measurements were overestimated by both raters (Figure 2.31). The Bland–Altman plot (Figure 2.32) for rater 1 shows that for lower volumes the mean difference was lower compared to that for higher flow volumes. The mean (SD) proportional difference in flow for rater 1 was 12.6 (7.7) % and for rater 2 was 7.8 (7.6) % (Figure 2.33).

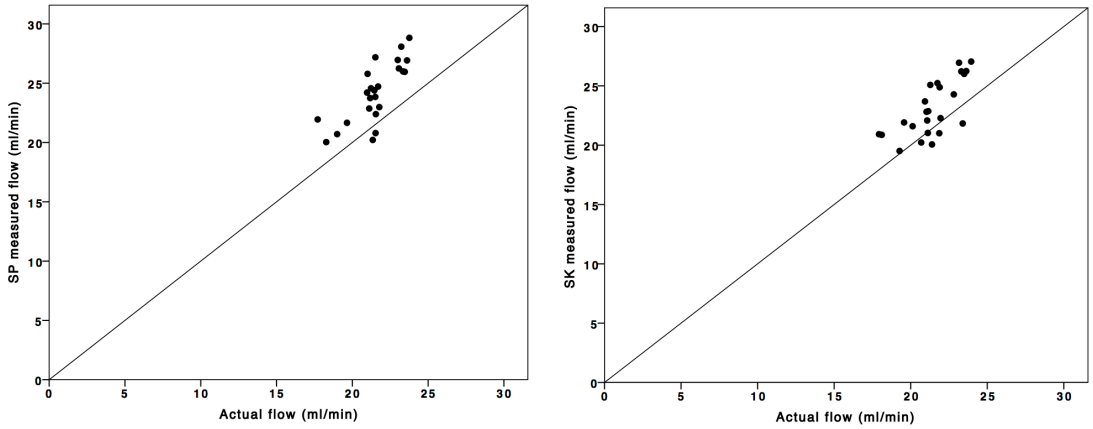


Figure 2.31: Correlation between flow volume measured and the actual measurements along with the line of equality.

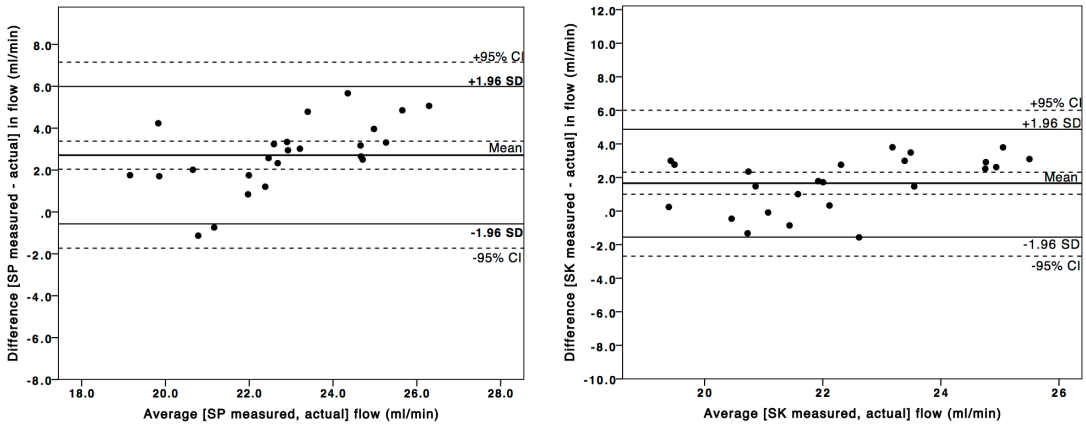


Figure 2.32: Bland–Altman plot of flow volume measurements for both raters.

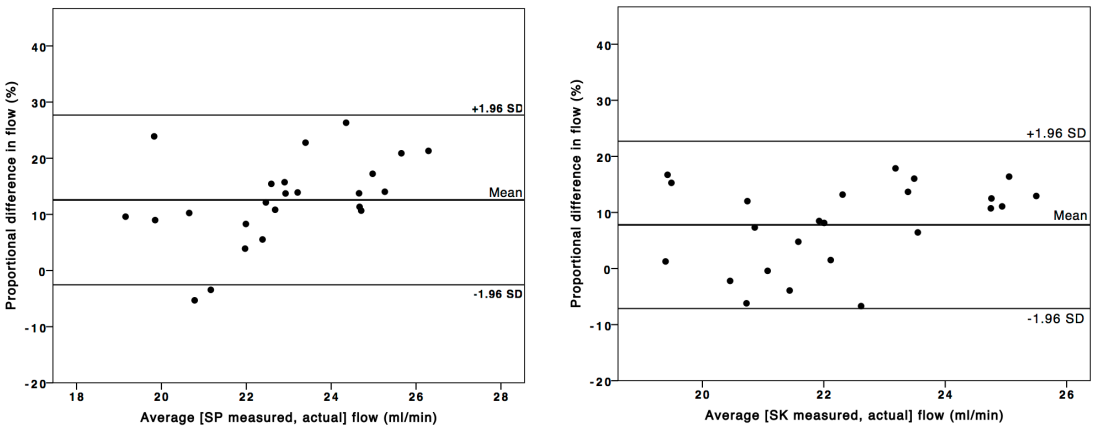


Figure 2.33: Plot illustrating the proportional difference in flow measurements for both raters.

Angle of insonation

The angle of insonation, which plays an important role in estimating the flow volumes, obtained for each of the measurements by the two raters were examined in addition to the diameter and flow measurements. The mean (SD, 95%CI) angle of insonation for rater 1 was 43.3 (3.9, 41.6 to 45.0) degrees and for rater 2 was 43.9 (4.7, 41.9 to 45.9) degrees. The relation between the two raters and difference between the two raters are shown in Figure 2.34.

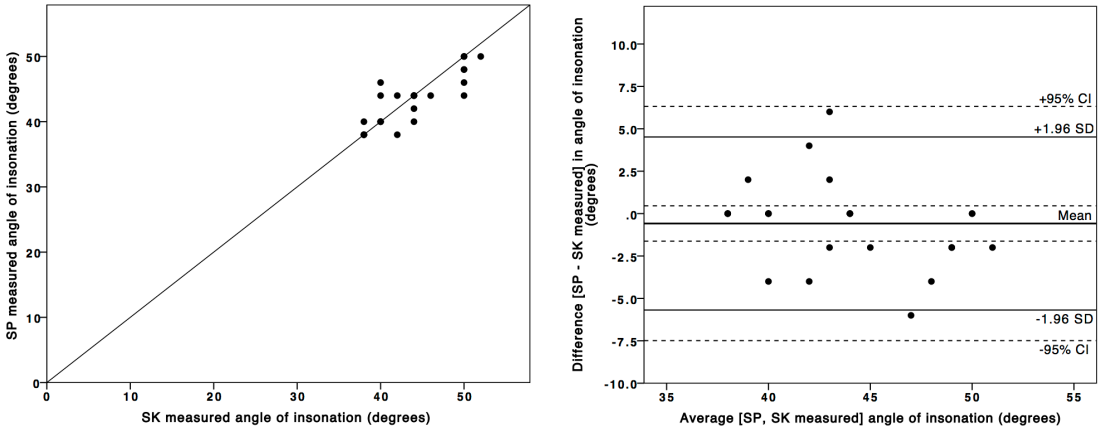


Figure 2.34: Correlation between angle of insonation along with line of equality and Bland–Altman plot between the two raters.

Inter rater reliability

Inter rater reliability was examined for vessel diameter, intensity weighted mean velocity (IWMV), angle of insonation and flow volume measurements for continuous and pulsatile flow. This analysis was performed using Intraclass correlation (ICC) using two-way mixed effects model ANOVA, with raters being fixed factors and measurements being random. The level of reliability between the raters was graded using previously described methods (Landis and Koch 1977).

Inter rater reliability (Table 2.3) using ICC(3,1) (Shrout and Fleiss 1979, Landers 2015) was 'almost perfect' (Landis and Koch 1977) for all measurements except pulsatile flow volume where it was 'moderate'.

The coefficient of variation [(SD/Mean)*100] was also calculated as an additional measure of agreement and results expressed as percentage. The coefficient of variation was calculated separately for each of the three groups of diameter measurements and the mean of these values were used. Similarly, the coefficient of variation for the high and low velocities (for continuous flow) were calculated and the mean of these values were used.

The inter rater coefficient of variation for vessel diameter measurements was 1.5%. The inter rater coefficient of variation for flow measurements between raters was 58.1%. This high value was due to flow measurements being varied in between measurements.

Type of flow	Parameter	Rater 1 (SP) Mean (SD, 95% CI)	Rater 2 (SK) Mean (SD, 95% CI)	Inter rater reliability (95% CI)
Continuous (n=18)	Vessel diameter (cm)	0.183 (0.032, 0.168 to 0.199)	0.185 (0.03, 0.168 to 0.2)	0.996 (0.990 to 0.999)
	IWMV (cm/s)	15.5 (7.5, 11.8 to 19.3)	15.8 (7.4, 12.1 to 19.5)	0.983 (0.955 to 0.993)
	Angle of insonation (θ)	43.7 (4.8, 41.3 to 46.1)	42.2 (4.8, 39.8 to 44.6)	0.839 (0.560 to 0.941)
	Flow volume (ml/min)	25.2 (14.1, 18.2 to 32.3)	26.1 (14.4, 18.9 to 33.3)	0.984 (0.957 to 0.994)
Pulsatile (n=24)	Vessel diameter (cm)	0.185 (0.03, 0.172 to 0.199)	0.185 (0.03, 0.171 to 0.199)	0.997 (0.993 to 0.999)
	IWMV (cm/s)	16.3 (6.2, 13.7 to 18.9)	15.7 (6.2, 13.1 to 18.3)	0.978 (0.938 to 0.991)
	Angle of insonation (θ)	43.3 (3.9, 41.6 to 45.0)	43.9 (4.7, 41.9 to 45.9)	0.822 (0.636 to 0.918)
	Flow volume (ml/min)	24.2 (2.5, 23.1 to 25.3)	23.1 (2.3, 22.1 to 24.1)	0.574 (0.218 to 0.793)

Table 2.3: Summary table showing the mean values and results of the inter rater reliability. SP–Sujith Pereira, SK–Steve Kempley, IWMV–Intensity weighted mean velocity.

Intra rater reliability

Intra rater reliability was performed for parameters measured in the continuous and pulsatile flow groups. Paired measurements of the vessel diameter, intensity weighted mean velocity (IWMV), angle of insonation and flow volume measurements from both rater were compared. As there were 18 measurements for continuous flow and 24 measurements for pulsatile flow, equal number of measurements from both flow types were chosen by eliminating the first set of measurements from the pulsatile flow group. The analysis was performed using Intraclass correlation (ICC) using two-way mixed effects model ANOVA, absolute agreement, with raters being fixed factors and measurements being a random factor. The level of reliability between the raters was graded using previously described methods (Landis and Koch 1977).

Intra rater reliability (Table 2.4) using ICC(3,1) (Shrout and Fleiss 1979, Landers 2015)

for both types was 'almost perfect' for the majority of parameters in both continuous and pulsatile flow parameters. Flow volume reliability was lesser for both raters compared to other parameters. For pulsatile flow, rater 1 had 'moderate' intra rater reliability whereas rater 2 had 'substantial' intra rater reliability.

In addition to ICC, the mean coefficient of variation [(SD/Mean)*100] was also calculated and results were expressed as percentage as a further test of agreement with raters. This was done for both the continuous and pulsatile flow groups.

In the continuous flow group, the intra rater coefficient of variation for vessel diameter measurements for rater 1 (SP) was 1.5% and that of rater 2 (SK) was 1.1%. The intra rater coefficient of variation for continuous flow measurements for rater 1 (SP) was 36.8% and that of rater 2 (SK) was 38.9%.

As for pulsatile flow group, the intra rater coefficient of variation for vessel diameter measurements within raters for rater 1 (SP) was 0.9% and that of rater 2 (SK) was 1.0%. The intra rater coefficient of variation for pulsatile flow measurements for rater 1 (SP) was 10.5% and that of rater 2 (SK) was 10.1%.

Test–retest reliability

Reliability testing frequently involves assessing test–retest reliability. It is increasingly being argued that this test is a measure of both validity and reliability (Berchtold 2016) and therefore I have calculated both of these for these measurement. Vessel diameter measurement measured on two separate occasions by both raters were compared to assess the

Type of flow	Parameter	Rater 1 (SP) Mean (SD, 95% CI)		Rater 1 (Intra) reliability (95% CI)	Rater 2 (SK) Mean (SD, 95% CI)		Rater 2 (Intra) reliability (95% CI)
		Measurement (a)	Measurement (b)		Measurement (a)	Measurement (b)	
Continuous (n=9)	Vessel diameter (cm)	0.183 (0.03, 0.158 to 0.209)	0.184 (0.03, 0.159 to 0.210)	0.998 (0.988 to 1.000)	0.185 (0.03, 0.160 to 0.211)	0.184 (0.03, 0.158 to 0.210)	0.999 (0.994 to 1.000)
	IWMV (cm/s)	21.4 (6.2, 16.7 to 26.2)	9.6 (2.2, 7.9 to 11.3)	-	21.5 (6.1, 16.7 to 26.2)	10.1 (2.5, 8.2 to 12.1)	-
	Angle of insonation (θ)	43.6 (4.5, 40.0 to 47.1)	43.8 (5.3, 39.7 to 47.9)	0.831 (0.557 to 0.972)	42.7 (5, 38.8 to 46.5)	41.8 (4.9, 38 to 45.6)	0.911 (0.675 to 0.979)
	Calculated flow (ml/min)	34.8 (13.7, 24.3 to 45.4)	15.6 (5.3, 11.5 to 19.7)	-	35.6 (13.9, 24.9 to 46.4)	16.6 (6.4, 11.6 to 21.5)	-
Pulsatile (n=12)	Vessel diameter (cm)	0.186 (0.03, 0.165 to 0.207)	0.185 (0.03, 0.164 to 0.205	0.998 (0.986 to 0.999)	0.185 (0.03, 0.164 to 0.207)	0.186 (0.03, 0.164 to 0.207)	0.998 (0.992 to 0.999)
	IWMV (cm/s)	16.2 (6, 12.3 to 20.0)	16.5 (6.6, 12.3 to 20.7)	0.972 (0.908 to 0.992)	15.4 (6.0, 11.6 to 19.2)	16.0 (6.7, 11.8 to 20.2)	0.980 (0.927 to 0.994)
	Angle of insonation (θ)	43.0 (3.6, 40.7 to 45.3)	43.4 (4.5, 40.8 to 46.5)	0.714 (0.273 to 0.908)	43.7 (4.7, 40.7 to 46.7)	44.2 (4.9, 41.0 to 47.3)	0.820 (0.492 to 0.944)
	Calculated flow (ml/min)	24.3 (2.4, 22.8 to 25.8)	24.1 (2.8, 22.3 to 25.9)	0.484 (-0.130 to 0.822)	22.7 (2.2, 21.3 to 24.1)	23.5 (2.5, 21.9 to 25.1)	0.749 (0.355 to 0.920)

Table 2.4: Summary table of the various parameters measured in both flows by both raters and the intra rater reliability. SP–Sujith Pereira, SK–Steve Kempley, IWMV–Intensity weighted mean velocity.

test–retest reliability and validity. The vessel diameter measurements were obtained from work done using continuous and pulsatile flow work which was performed few months apart. As the flow between the two experiments were varied, the IWMV and the angle of insonation were not considered for test–retest reliability work.

No.	Chamber Number	Rater 1 (SP) measurement (i) diameter (cm)	Rater 1 (SP) measurement(ii) diameter (cm)	Difference SP [i-ii] diameter (cm)	Average SP [i,ii] diameter (cm)	Rater 2 (SK) measurement (i) diameter (cm)	Rater 2 (SK) measurement (ii) diameter (cm)	Difference SK [i-ii] diameter (cm)	Average SK [i,ii] diameter (cm)
1	A1	0.144	0.144	0	0.144	0.149	0.148	0.001	0.1485
2	A1	0.145	0.145	0	0.145	0.148	0.145	0.003	0.1465
3	A2	0.147	0.146	0.001	0.1465	0.149	0.144	0.005	0.1465
4	A2	0.149	0.146	0.003	0.1475	0.147	0.146	0.001	0.1465
5	A3	0.148	0.148	0	0.148	0.146	0.145	0.001	0.1455
6	A3	0.148	0.144	0.004	0.146	0.145	0.144	0.001	0.1445
7	B1	0.178	0.191	-0.013	0.1845	0.179	0.187	-0.008	0.183
8	B1	0.178	0.188	-0.010	0.183	0.18	0.187	-0.007	0.1835
9	B2	0.182	0.191	-0.009	0.1865	0.185	0.184	0.001	0.1845
10	B2	0.186	0.186	0	0.186	0.183	0.189	-0.006	0.186
11	B3	0.182	0.186	-0.004	0.184	0.185	0.185	0	0.185
12	B3	0.184	0.187	-0.003	0.1855	0.182	0.186	-0.004	0.184
13	C1	0.227	0.225	0.002	0.226	0.224	0.228	-0.004	0.226
14	C1	0.226	0.225	0.001	0.2255	0.226	0.227	-0.001	0.2265
15	C2	0.222	0.223	-0.001	0.2225	0.226	0.222	0.004	0.224
16	C2	0.224	0.222	0.002	0.223	0.226	0.226	0	0.226
17	C3	0.219	0.221	-0.002	0.22	0.223	0.225	-0.002	0.224
18	C3	0.220	0.219	0.001	0.2195	0.221	0.222	-0.001	0.2215
Mean		0.184	0.185	-0.002	0.185	0.185	0.186	-0.001	0.185
SD		0.032	0.032	0.005	0.032	0.032	0.034	0.004	0.033

Table 2.5: Vessel diameter measurement values taken on two different occasions by both raters. SP–Sujith Pereira, SK–Steve Kempley.

Two measurements from all the nine chambers that were taken by both raters (Table 2.5) were examined to ensure equal measurement numbers for each rater. This was achieved by including the first two pairs of measurements and discarding the third measurement that was done for some chambers during the pulsatile flow arm of the experiment.

The mean (SD) difference in vessel diameter measurements for rater 1 was -0.002 (0.005)

cm and that for rater 2 was -0.001 (0.004) cm. The vessel diameter measurements from both raters were compared (Figure 2.35) and a Bland–Altman plot was used to asses the mean difference along with the limits of agreement for both raters (Figure 2.36).

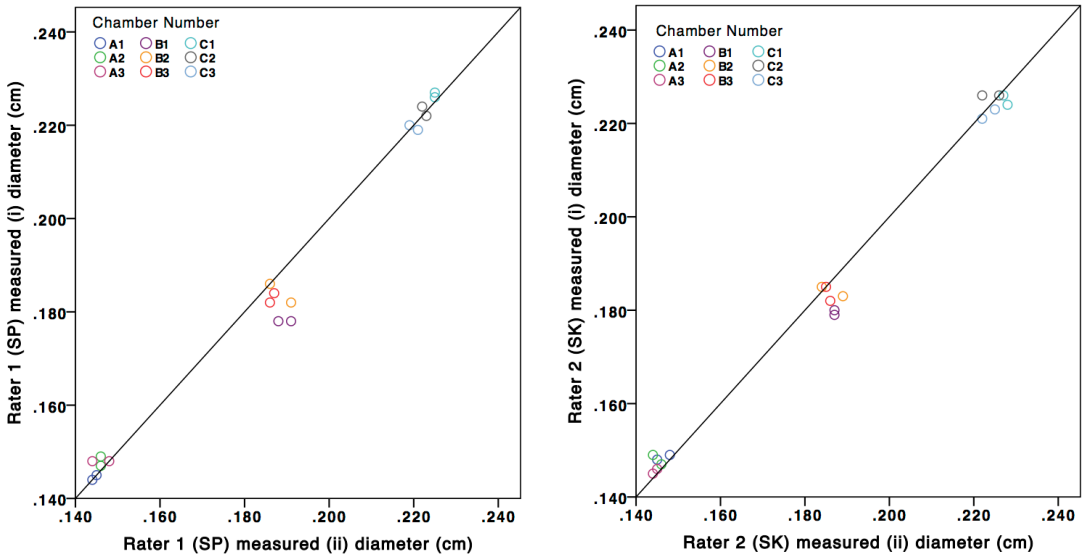


Figure 2.35: Correlation between the two measurements performed on two occasions by the two raters along with line of equality.

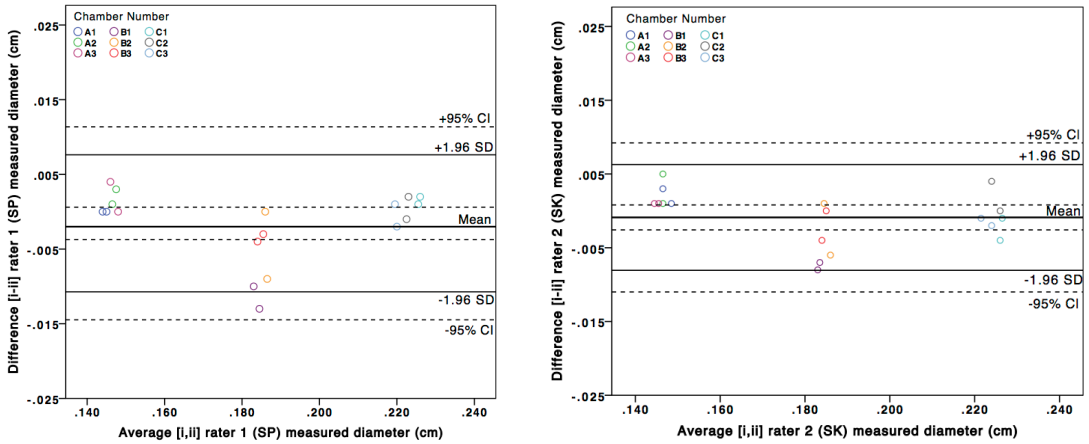


Figure 2.36: Bland–Altman plot illustrating the limits of agreement between two measurements performed by both raters.

Test–retest reliability for both the raters was 'almost perfect' (Table 2.6) with a reliability of 0.989 and 0.994 for rater 1 and rater 2 respectively.

Rater	Measurement (i) Mean (SD, 95% CI)	Measurement (ii) Mean (SD, 95% CI)	Test–retest reliability (95% CI)
1 (SP)	0.184 (0.03, 0.168 to 0.199)	0.185 (0.03, 0.170 to 0.201)	0.989 (0.971 to 0.996)
2 (SK)	0.185 (0.03, 0.168 to 0.200)	0.186 (0.03, 0.169 to 0.202)	0.994 (0.984 to 0.998)

Table 2.6: Mean vessel diameter measurements and test–retest reliability statistics.

Discussion of flow phantom studies

We have designed and constructed a flow phantom model of the common carotid artery which produces both continuous and pulsatile flow mimicking the physiological flow patterns seen in extremely preterm infants.

The results of the phantom model gave useful information of techniques that can improve the measurement of flow volumes. Firstly, the use of a gel wedge has shown to reduce the angle of insonation and capture low velocity flow. Secondly, gain optimisation during measurement of the vessel diameter will reduce significant errors due to the squaring whilst calculating flow volumes. Thirdly, maintaining the wall filter at minimum will capture all the low velocity flow and avoid over-estimation of flow volumes. Lastly, the use of steerable angle can help in achieving the lowest insonation angle and more accurate flow volume estimation.

There was very good inter rater and intra rater reliability for the majority of measurements using continuous and pulsatile flow. The intra rater agreement for flow volume was comparably lower for both raters. However when the measurements used to calculate the flow volume were investigated separately, there was very good intra reliability for both raters. As for continuous flow, intra rater reliability was poor because the velocities were altered

during the experiment. Test–retest measurements showed that these were valid with very good reliability.

The inter rater coefficient of variation for vessel diameter measurement was 1.5%, which was lower than the 3.9% reported by Sinha et al (Sinha et al. 2006). The intra rater coefficient of variation for vessel diameter measurements for rater 1 was 1.5% and for rater 2 was 1.1%. The intra rater coefficient of variation for pulsatile flow measurements for rater 1 was 10.5% and that for rater 2 was 10.1%. These values were comparable to 10.5% and 15.4% for the two raters reported by Sinha and colleagues (Sinha et al. 2006). The intra rater coefficient of variation for pulsatile flow vessel diameter measurements for rater 1 was 0.9% and that for rater 2 was 1.0%. These values were lower than the one obtained when using continuous flow which may reflect the raters becoming more familiar with the equipment as the pulsatile flow measurements were done a few months later than the continuous flow measurements.

Validity work on the vessel diameter measurements revealed an underestimation of around 7% (95%CI -12% to -4%) and 7% (95%CI -13% to -0.5%) for continuous and pulsatile flow respectively. This means that we would expect 95% of the repeated diameter measurements to be underestimated by about 0.5–15% of the first measurement. On comparing flow volume measurements, we found that this was overestimated by approximately 12% using continuous flow when compared to pulsatile flow where it was lower at 7%. This is well within the 30% acceptance limit which was found from a large meta–analysis of twenty-five studies examining bias and precision statistics when comparing cardiac output measurements using different techniques (Critchley and Critchley 1999).

Our work examining vessel diameter measurements showed the presence of a systemic error with underestimation of the diameter by both raters. This could be explained by the material used for the vessel. Polytetrafluoroethylene (PTFE) tubing of varying diameter was used to ensure it maintained its diameter throughout the entire length of the tube whilst being embedded in the tissue mimicking material. PTFE being denser than human tissue is prone to acoustic attenuation. Alternative tubing which would be more similar to human tissue would be silicone, but this was prone to losing its circular shape. Hence, for this model, PTFE tubing was preferred. The large hyper echoic shadow produced by PTFE on cross–sectional view could possibly be a cause for error in estimation of the precise diameter. This could be due to difficulties by the raters in estimating the mid–point of the vessel. Alternative solution to the issue of attenuation associated would be to consider wall–less phantom models (Kenwright et al. 2015).

This flow phantom is a realistic in vitro model of the common carotid artery of extremely preterm infants and which could provide useful information on fluid dynamics and help to train clinicians in measuring flow volumes involving very small calibre vessels. The model could be further improved with the use of an wall–less model where errors associated with acoustic attenuation could be eliminated thus improving accuracy.

Chapter 3

Results

3.1 Relationship between cerebral blood flow, cardiac output and blood pressure

The aim of this work was to investigate the relationship between the physiological parameters measured in this study. Here we compare the relationship between right common carotid artery blood flow, cardiac output and BP in extremely premature newborn infants.

Patient characteristics

Sixty infants were studied. Mean (range) gestation and birthweight were 25.8 (23.3 –28.9) weeks and 817 (470 –1470) grams respectively. This cohort consisted of thirty males (50%). The majority of infants received ventilatory support. (56/60 (93%) of infants on day 1 and 43/60 (72%) of infants on day 3 of postnatal life). The mean (SD) $PaCO_2$ on day 1 was 5.2 (1.5) KPa and on day 3 was 5.6 (1.1) KPa.

The scans were performed on day 1 at a median age of 18 hours (IQR 12–22) and on day 3 at a median age of 77 hours (IQR 69–91). The mean (SD) RCCA diameter on day 1 was 1.73 (0.3) mm and on day 3 was 1.71 (0.3) mm. The mean (SD) RCCA diameter was 1.78 (0.2) mm for males and 1.64 (0.4) mm for females. RCCA diameter was significantly related to gestation and birth weight (Figure 3.1).

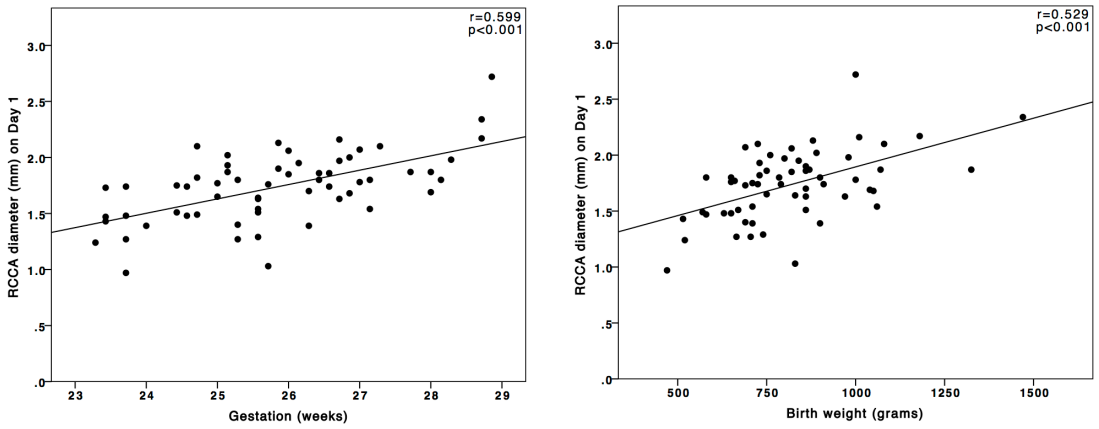


Figure 3.1: Scatter plot illustrating the direct relationship between gestation and birth weight with RCCA diameter on day 1 of postnatal life. Pearson's correlation used.

Right common carotid artery flow volumes increased significantly with a rise in gestational age (Figure 3.2). The median (IQR) RCCA flow volume increased significantly (p=0.001, Wilcoxon rank test) from 12 (9–15) ml/kg/min on day 1 of postnatal life to 14 (12–18) ml/kg/min on day 3 of postnatal life (Figure 3.3). The mean RCCA flow without adjusting for birth weight in this cohort was 16 ml/min on day 1 and 19ml/min on day 3.

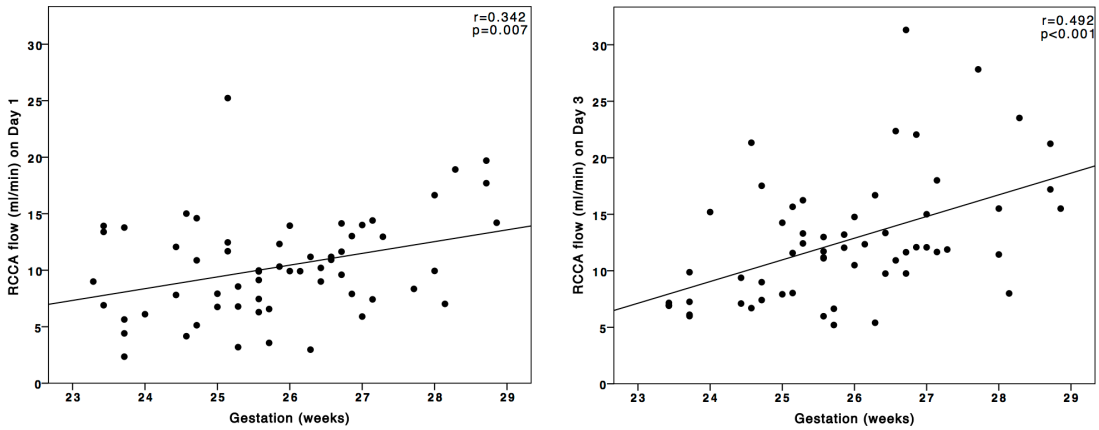


Figure 3.2: Scatter plot illustrating the direct relationship between gestational age and right common carotid artery flow. Pearson's correlation used.

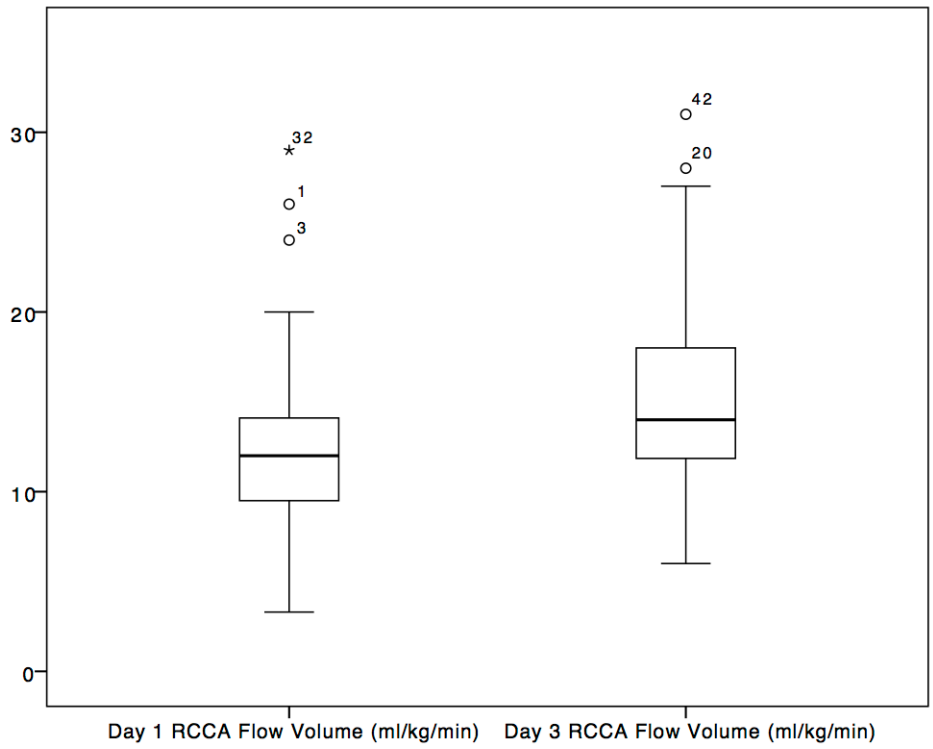


Figure 3.3: Box plot illustrating significant increase in RCCA flow volumes between day 1 and 3 of postnatal life. (p=0.001)

Median (IQR) cardiac output increased significantly (p<0.001, Paired t–test) from 166 (144–203) ml/kg/min on day 1 of postnatal life to 211 (161–250) ml/kg/min on day 3 of postnatal life (Figure 3.4). There was no statistically significant relationship between RCCA flow volumes and cardiac output on both days. There was a very weak non-significant positive association between RCCA and cardiac output on day 1 only with no relation on day 3 of postnatal life (Figure 3.5). When examining this relationship in infants whose PDA were closed, there was a positive association between RCCA and cardiac output on both days which was not statistically significant (Figure 3.6).

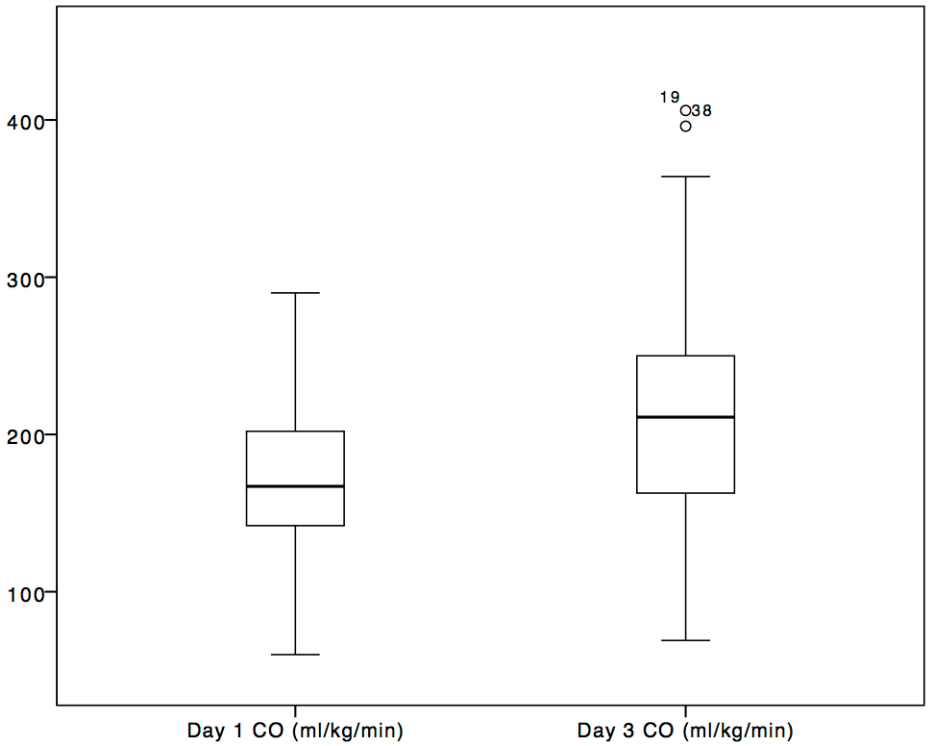


Figure 3.4: Box plot illustrating significant increase in cardiac output between day 1 and 3 of postnatal life. (p=0.001)

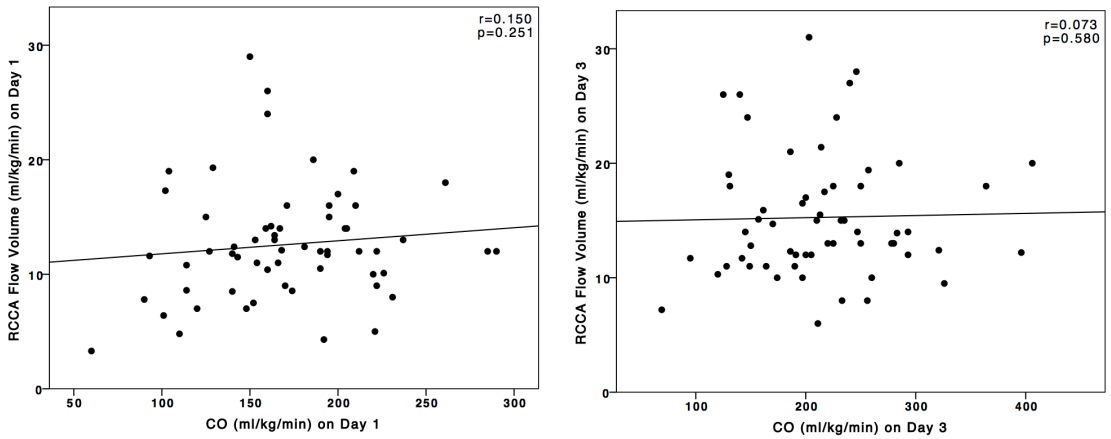


Figure 3.5: Scatter plot illustrating correlation between RCCA and CO on day 1 and 3 of postnatal life. (Spearman's Rho used)

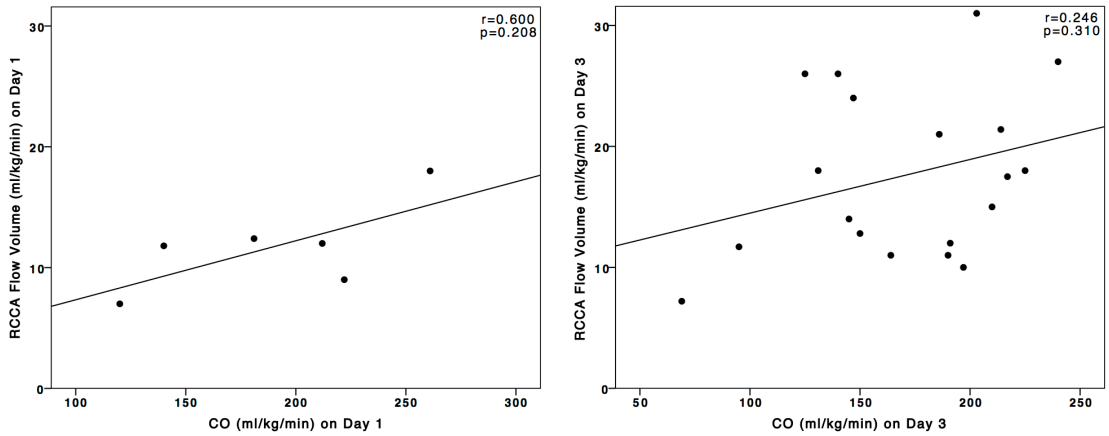


Figure 3.6: Scatter plot illustrating correlation between RCCA and CO on day 1 and 3 of postnatal life in infants with a closed PDA. (Spearman's Rho used)

There was no statistically significant relationship between mean arterial (invasive) BP and cardiac output. BP was negatively associated with cardiac output on day 1 (Figure 3.7) or day 3 (Figure 3.8) of postnatal life.

On day 1, 11/50 (22%) infants had a normal BP but low cardiac output and 9/50 (18%) infants had a low BP but normal cardiac output. In comparison, these values were lower on day 3 where 4/40 (10%) infants had a normal BP but low cardiac output and 5/40 (12%) infants had a low BP with normal cardiac output.

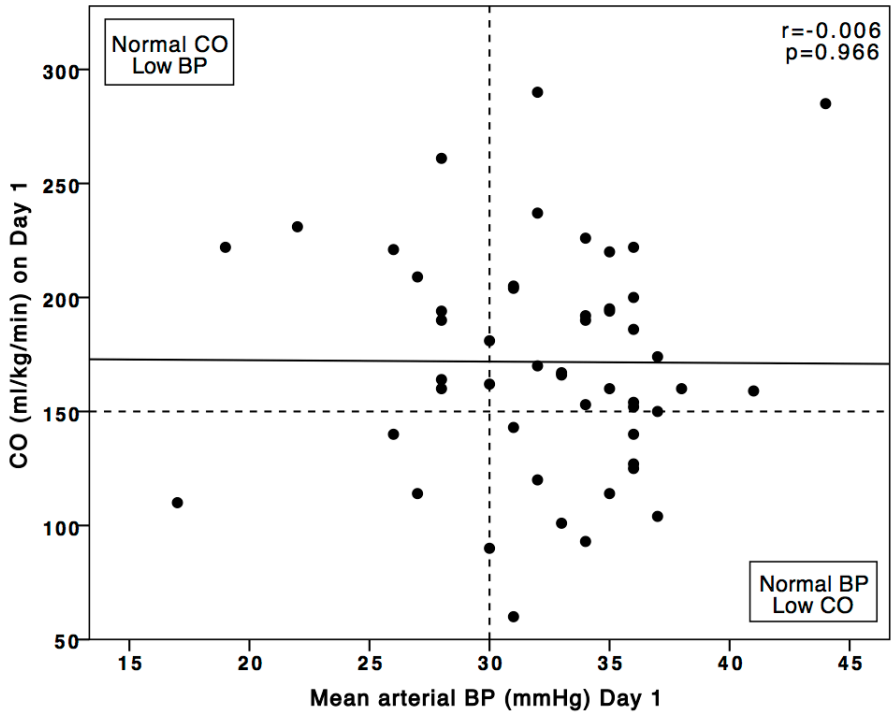


Figure 3.7: Scatter plot illustrating correlation between CO and invasive mean arterial BP on day 1 of postnatal life (Pearson's correlation used). Solid line represents line of fit and dotted lines represent normal limits of cardiac output and BP.

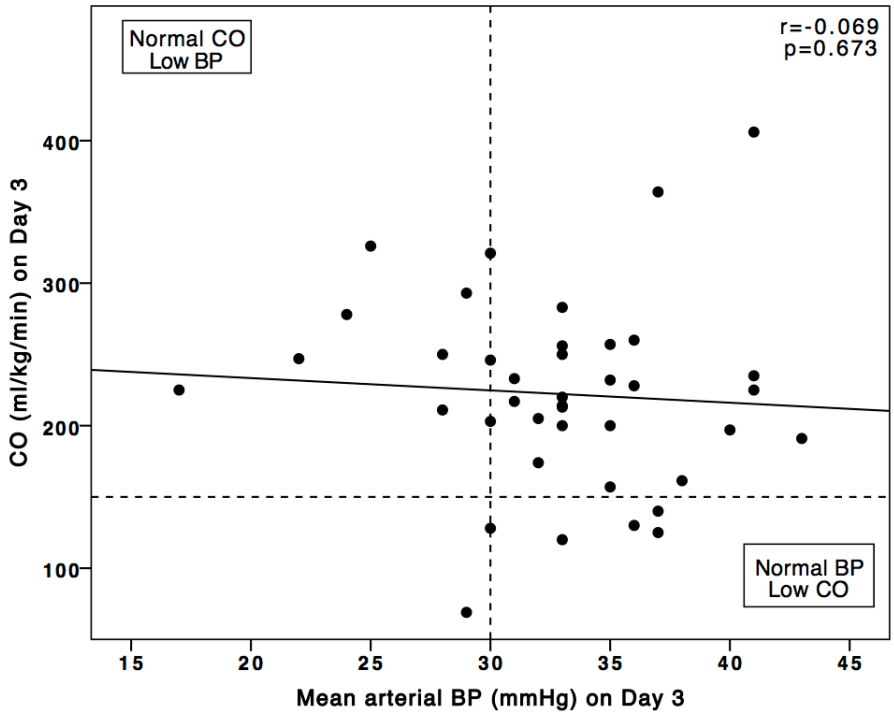


Figure 3.8: Scatter plot illustrating correlation between CO and invasive mean arterial BP on day 3 of postnatal life (Pearson's correlation used). Solid line represents line of fit and dotted lines represent normal limits of cardiac output and BP.

Systemic vascular resistance (mmHg/L/min/kg) was derived by dividing the BP by the cardiac output. The mean (SD) systemic vascular resistance on day 1 of postnatal life was 215 (79) mmHg/L/min/kg on day 3 was 196 (93) mmHg/L/min/kg. Systemic vascular resistance did not significantly change between day 1 and 3 (p=0.084) (Figure 3.9). There was no relationship between systemic vascular resistance and RCCA flow volumes both on day 1 and 3 (Figure 3.10 and 3.11) .

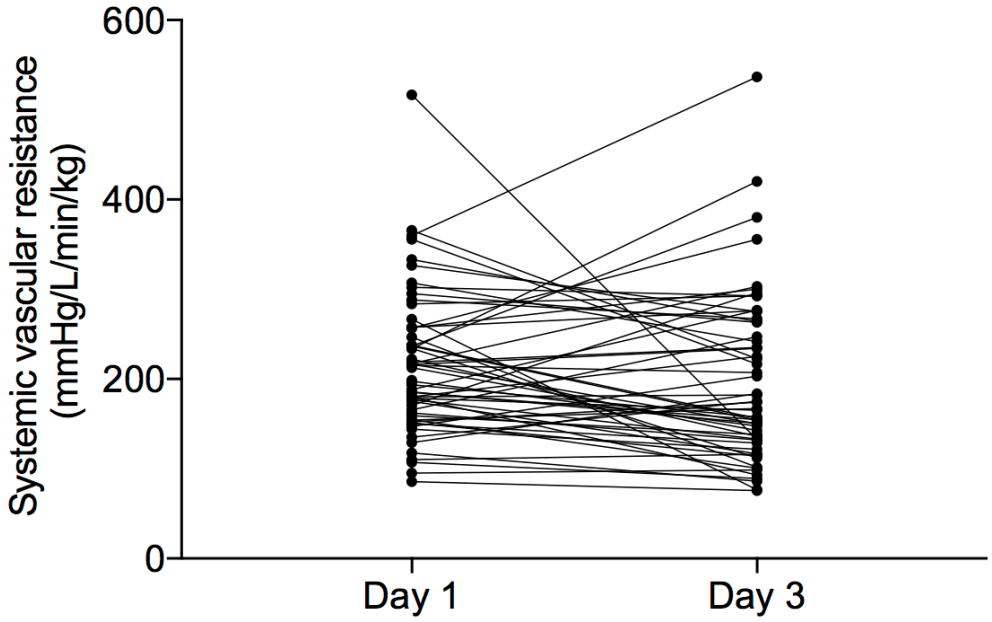


Figure 3.9: Systemic vascular resistance did not change significantly between day 1 and 3 of postnatal life.

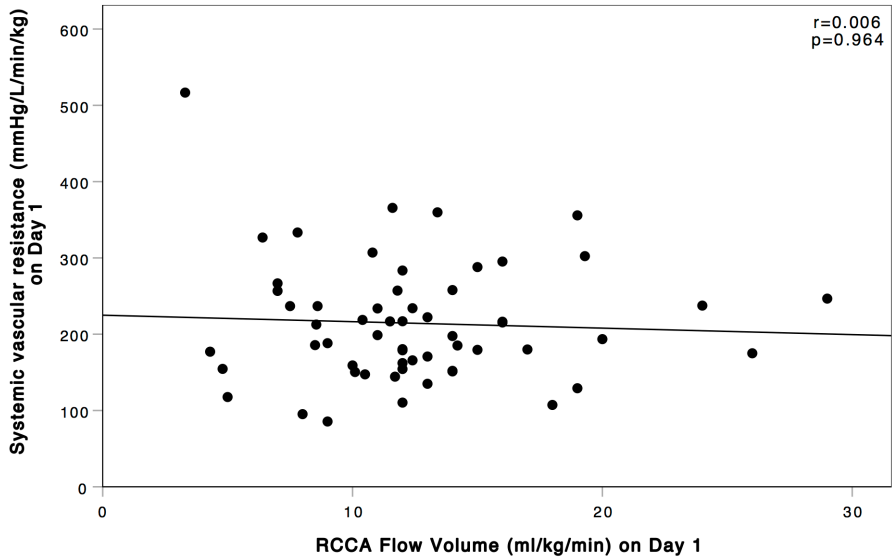


Figure 3.10: Scatter plot illustrating correlation between systemic vascular resistance and RCCA flow volume on day 1 of postnatal life. (Spearman's rho used)

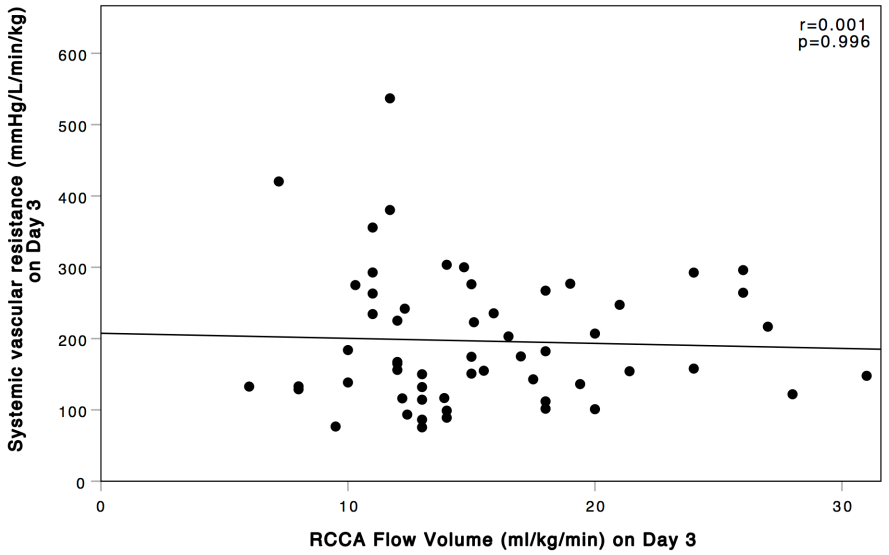


Figure 3.11: Scatter plot illustrating correlation between systemic vascular resistance and RCCA flow volume on day 3 of postnatal life. (Spearman's rho used)

Mean arterial BP (mixture of invasive and non–invasive) was significantly related to RCCA flow volumes on day 1 of postnatal life (Spearman's rho, r=0.35, p=0.007) only. This relationship was not significant on day 3 of postnatal life (Spearman's rho, r=0.16, p=0.227) (Table 3.1).

Further analysis was carried out with inclusion of infants with invasive only BP and in-

fants with invasive only BP and a haemodynamically insignificant PDA (closed or ductal diameter < 1.5 mm) (Figures 3.12 and 3.13). This resulted in the relation between RCCA flow volumes and BP being more stronger to a significant level. (Table 3.1) .

	RCCA flow on Day 1	
	Spearman's rho, r	p value
Mean arterial BP (invasive and non-invasive)	0.35	**0.007**
Mean arterial BP (invasive only)	0.31	**0.030**
Mean arterial BP (invasive only) and haemodynamically insignificant PDA	0.34	**0.020**
	RCCA flow on Day 3	
	Spearman's rho, r	p value
Mean arterial BP (invasive and non-invasive)	0.16	0.227
Mean arterial BP (invasive only)	0.39	**0.014**
Mean arterial BP (invasive only) and haemodynamically insignificant PDA	0.49	**0.012**

Table 3.1: Table showing the correlation between RCCA flow and BP on day 1 and 3 of postnatal life.

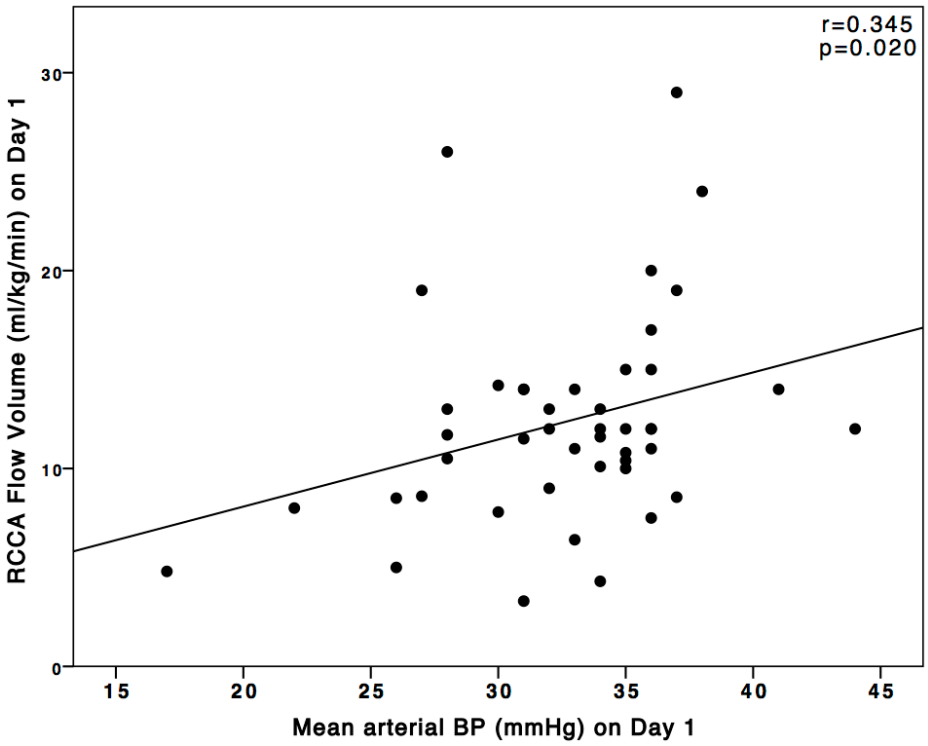


Figure 3.12: Scatter plot illustrating correlation between RCCA and invasive mean arterial BP on day 1 of postnatal life in infants with a haemodynamically insignificant PDA. (Spearman's rho used)

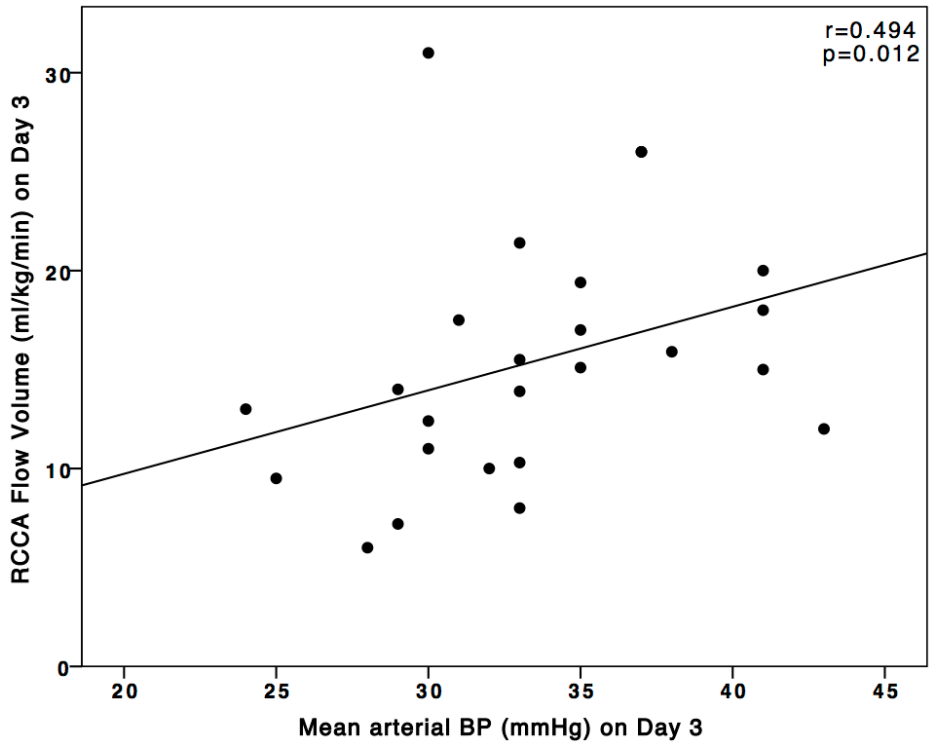


Figure 3.13: Scatter plot illustrating correlation between RCCA and invasive mean arterial BP on day 3 of postnatal life in infants with a haemodynamically insignificant PDA. (Spearman's rho used)

The mean (SD) percentage of cardiac output that was directed to the right common carotid artery was 7.84% (3.7) on day 1 and 7.87% (3.8) on day 3 of postnatal life. Assuming equal contribution to cerebral blood flow from the right and left common carotid arteries, approximately 16% of the cardiac output is distributed to both the carotid arteries. The percentage of cardiac output (mean of day 1 and day 3 of postnatal age) that contributed to the RCCA blood flow volume was not statistically significant (Figure 3.14).

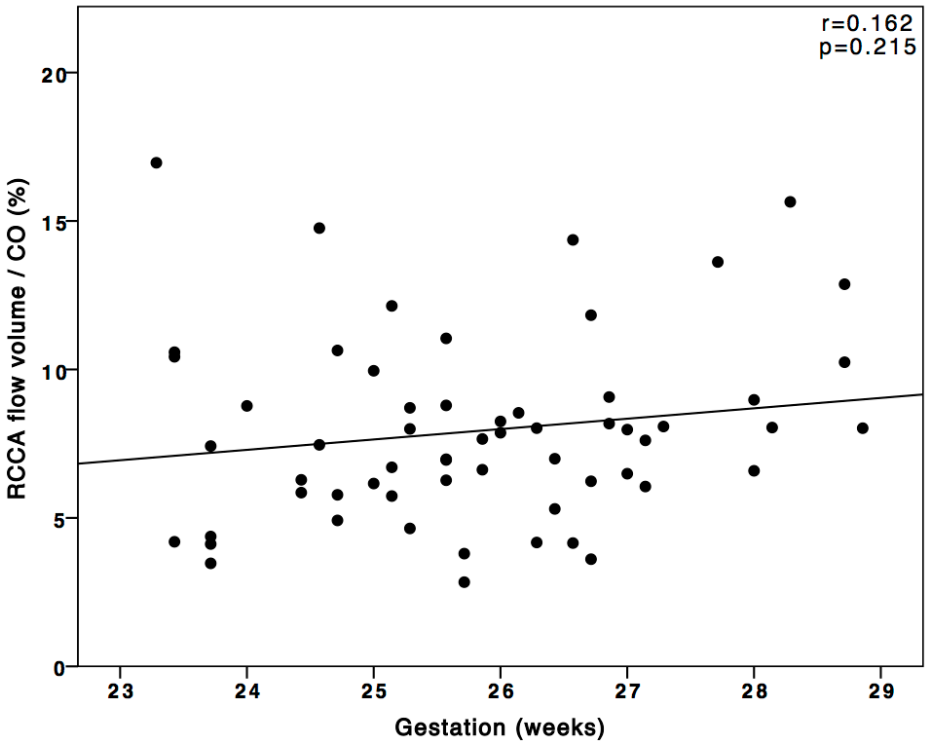


Figure 3.14: Percentage of RCCA to CO (mean of day 1 and 3) did not differ with gestation. Pearson's correlation used.

3.2 Relationship between cerebral blood flow, cardiac output and blood pressure - discussion

3.2.1 Cerebral blood flow

The right common carotid artery diameter measurements were directly related to gestation and birth weight. The mean (SD) RCCA diameter for males and females were 1.78 (0.2) mm and 1.64 (0.4) mm respectively in this cohort. These measurements were slightly lower than work done by Sehirli and colleagues (Sehirli et al. 2005) who examined post-mortem samples of the right common carotid artery diameter measurements in 20 infants with a gestation of 34 to 40 weeks. They found the mean (SD) right common carotid artery diameter measurements for males to be 1.95 (0.3) mm and for females to be 1.78 (0.3) mm. The smaller diameter of the RCCA in our cohort is accounted for by the lower gestational age studied in this cohort.

As expected, cerebral blood flow increased significantly with gestational age and birth weight (Kehrer et al. 2002). The right common carotid artery blood flow volumes in this cohort rose significantly from day 1 of postnatal life to day 3 of postnatal life. The right common carotid artery blood flow volumes in this study were lower (mean value of 15ml/kg/min vs 20ml/kg/min) when compared to those obtained by Sinha and colleagues (Sinha et al. 2006) using the same method to measure cerebral blood flow. This difference could be explained by several reasons; firstly the lower gestational age and birthweight of infants (mean gestation of 25.8 versus 33.2 weeks and mean birthweight of 817 versus 1450 grams), secondly the effect of mechanical ventilation; almost all the infants in this study were mechanically ventilated at the time of measurement as opposed to none of the infants being ventilated in the other study. Previous work has shown that mechanical ventilation (Oskar Baenziger 1994, Greisen 1986) and the mode of ventilation (Milan et al. 2009) can reduce cerebral blood flow. Thirdly, infants in this cohort were 'sicker' when compared to the more stable and more mature preterm infants studied by Sinha et al (Sinha et al. 2006). Raju and colleagues (Raju et al. 1987) examined a small number of preterm infants found the RCCA flow volume to be 32 ml/min, which is higher than the values obtained in this study (mean value of 16 ml/min on day 1 and 19 ml/min on day 3). The use of a 7.5 MHz transducer which had fixed sampling gate could explain the higher values that they obtained. Given the small diameters that were being measured using this transducer would lead to a larger variability in diameter measurements when compared to the higher 15 MHz transducer that was used in this study. Kehrer et al (Kehrer et al. 2002) examined more mature infants using the internal carotid artery and vertebral artery using a 10 MHz transducer. They found the median cerebral blood flow to be around 33 ml/min which increased with gestation, a finding replicated in this cohort. Though close comparison is made with the work carried out by Sinha and colleagues (Sinha et al. 2006)

who used the same method for measurement of cerebral blood flow, other groups (Meek et al. 1998, Noone et al. 2003) also examined infants of similar gestation but used near infrared spectroscopy and found similar conclusions of increase in cerebral blood flow during the first three days of postnatal life. In this study, the proportion of cardiac output that contributed to the cerebral blood flow (assuming equal contribution from both carotid arteries) was 16% which is comparable with the 15-25% of cardiac output previously reported (Kandel et al. 2012, Sato et al. 2011, Sinha et al. 2006, Magistretti 1999).

RCCA flow volumes were not correlated with systemic vascular resistance both on day 1 and 3 which may could be due to several reasons including the role of patent ductus arteriosus which can reduce the systemic vascular resistance and the complex phenomenon of cerebral autoregulation.

The validity and reliability of measurement of cerebral blood flow using the carotid artery was examined using a flow phantom model that was created using data captured from premature infants randomly who were admitted to the neonatal unit. Microvessel flow phantom models exist for investigating intracerebral blood flow in preterm infants (Camfferman et al. 2014). To our knowledge, this is the only flow phantom model of the carotid artery of the extremely preterm infant. Validity work found that vessel diameter measurements were underestimated by nearly 7% by both raters, which was well within acceptable limits (Critchley and Critchley 1999). This degree of error was due to the acoustic attenuation caused by the vessel wall which is far higher than human tissue resulting in a hyper echoic signal, thus making diameter estimation challenging. Though this has the potential to cause a significant error in flow volumes due to the squaring effect, this could be reduced by taking an average of several readings of the diameter. In future studies, this

model could be improved by using a tubing that is more similar to human tissue and firm to maintain its circular diameter in the tissue mimicking material. Other alternative options would be to create a wall–less flow phantom using agar based tissue mimicking material that conducts the speed of sound at 1548 m/sec (Sun et al. 2012), similar to human tissue, and thus making diameter measurements more accurate. Nevertheless, there was very good inter and intra rater reliability, for continuous and pulsatile flow, for measurement of the vessel diameter, intensity weighted mean velocity and angle of insonation. The reliability results from this cohort was comparable to the work carried out by Camfferman and colleagues (Camfferman et al. 2014) who performed similar studies using microvessel flow phantom to investigate the intracerebral circulation. In contrast to underestimation of vessel diameter measurements, they systematically overestimated the vessel diameter. The coefficient of variation for vessel diameter measurements (1.5% versus 3%) and intra rater observations (10% versus 15%) were comparable to (Sinha et al. 2006). The test–retest reliability examining vessel diameter only was also acceptable making this a useful tool to interrogate the cerebral blood flow using the carotid artery in this group of infants.

3.2.2 Cardiac output

Cardiac output (median) increased significantly from 166 ml/kg/min on day 1 to 211 ml/kg/min on day 3 of postnatal life. These values were comparable to other studies (Kissack et al. 2005, M. Kluckow 1996, Pladys et al. 1999, Victor, Appleton, Beirne, Marson and Weindling 2006). Kluckow et al (M. Kluckow 1996) studied sixty-seven infants with a median gestation and birthweight of 28 weeks and 1015 grams respectively. They found median cardiac outputs of 170 ml/kg/min to 217 ml/kg/min for infants with mean BP of less and more than 30 mmHg respectively. Pladys et al (Pladys et al. 1999) who studied 17

infants with a gestational age of 27 to 30 weeks, found cardiac output to be 185 ml/kg/min in a proportion of infants with low BP. Victor et al (Victor, Appleton, Beirne, Marson and Weindling 2006) investigated 40 infants with a median gestation and birth weight of 27 weeks and 947 grams respectively. They reported the median left ventricular output to be 190 ml/kg/min in the first 48 hours of age. Sirc et al (Sirc et al. 2013) studied 22 preterm infants with a mean gestation and birth weight of 25.9 weeks and 852 grams. They reported the left ventricular output to vary from 190 ml/kg/min to 280 ml/kg/min in the first 48 hours of life.

In this cohort, the left ventricular output was used to represent the cardiac output. In this group of extremely premature infants, due to the persistence of fetal channels, some critics may argue that the left ventricular output may over–estimate the cardiac output due to shunting across the ductus arteriosus (Evans and Iyer 1994). Using the right ventricular output is also met with issues such as shunting across the atrium. More recent work considered using superior vena cava as a surrogate of systemic flow and more importantly cerebral blood flow (Kluckow and Evans 2000*b*) mainly because it is free from shunting and assumed to be more accurate. As the superior vena cava is cresenteric in shape (Ficial et al. 2013) rather than a circular, calculating flow volumes using the formula for a circular structure would be misleading and this was one of the limitations of using the superior vena cava to estimate systemic flow. There has been further work on assessment of systemic blood flow using a modified way of measuring the superior vena caval flow (Ficial et al. 2017).

3.2.3 Cerebral blood flow and cardiac output

We found a non–significant weak positive association between RCCA blood flow volume and cardiac output on day 1 and no relation on day 3 in this cohort. This relationship was stronger, but remained non–significant, when analysis was performed using infants who had a closed PDA. The lack of a significant relationship between cerebral blood flow and left ventricular output found in our study is consistent with other studies. Victor et al (Victor, Appleton, Beirne, Marson and Weindling 2006) using cerebral fractional extraction of oxygen and electroencephalography as a surrogate of cerebral perfusion and cerebral blood flow, found that no relationship existed between these and left ventricular output in the first few days after birth. Further work by Sirc and colleagues (Sirc et al. 2013) who used superior vena cava flow to represent cerebral blood flow found a correlation early on with left ventricular output only at 6 and 12 hours of age but not beyond that.

The lack of a consistent and significant relationship between left ventricular output and carotid artery blood flow in this cohort of infants could be due to the following reasons; firstly, the presence of PDA which could increase the left ventricular output (Evans and Iyer 1994) especially after 24 hours of age when ductal shunting becomes more significant. This is supported by Sirc et al (Sirc et al. 2013) who showed very little difference in the correlation between superior vena caval flow with RVO and LVO in the first 12 hours. Secondly, measurement of the carotid blood flow at fixed points on day 1 and day 3 of postnatal life may not have picked up this true relationship. Thirdly, measurement errors due to the small diameter of the carotid artery in this group of infants could easily lead to erroneous estimation of the cerebral blood flow especially due to the squaring that is done to calculate the cerebral blood flow. Lastly, the complex relation between cerebral autoregulation and various physiological parameters influencing it could play a role in the

lack of relation seen in this cohort of infants (Boylan et al. 2000, Jayasinghe et al. 2003, Fenton et al. 1992).

Cerebral blood flow measured using the right common carotid artery was found to be approximately 8% of the cardiac output. Assuming equal contribution from both carotid arteries, this would equate to a total of 16% of the cardiac output contributed to cerebral blood flow, a finding comparable to other studies as discussed earlier. This was lower than Sinha et al (Sinha et al. 2006) who found that 11% of the cardiac output was distributed to the right common carotid artery (22%, assuming equal contribution from both the carotid arteries). This difference can be explained by several factors; scans were done on day 1 and 3 in this cohort which included infants of a lower gestation who were 'sicker' and ventilated as opposed to Sinha et al where the preterm infants were not ventilated with median age of scanning at 9 days. Majority of the infants in our study would have a patent ductus arteriosus which will lower the mean arterial BP owing to lower systemic vascular resistance and falsely elevate the left ventricular output due to increased venous return secondary to ductal shunting.

3.2.4 Cardiac output and blood pressure

In this cohort of infants, cardiac output (left ventricular output) and BP were found to have an negative correlation, a finding previously described (Pladys et al. 1999). BP, a complex variable, is the result of interplay of multiple factors. It is dependent on the venous return (preload), myocardial contractility, systemic vascular resistance (after load) and pulmonary vascular resistance as well. Venous return (preload) is influenced by several factors such as gestation, volume loss, level of ventilatory support including mode of ventilation, poorly replaced insensible loss and third spacing of fluid. Myocardial con-

tractility varies depending on the preterm myocardium, which is not designed to support the systemic circulation at this gestation. Sepsis and hypoxia have a negative impact on myocardial contractility. Systemic vascular resistance (after load) is dependent on the gestation and the use of inotropes which predominantly have a peripheral vasoconstrictive effect. Pulmonary vascular resistance is increased in the first few days after birth and the presence of a PDA can influence the BP. Hence, it is not surprising that several studies comparing the relationship between BP and cardiac output have conflicting reports (M. Kluckow 1996, Walther et al. 1994, Munro et al. 2004, Tyszczuk et al. 1998, Meek et al. 1999, Wardle et al. 1999, Gill and Weindling 1993).

On day 1, 22% of infants had a normal BP with low cardiac output and 18% of infants had normal cardiac output with low BP. These were comparable to values found by Kluckow and colleagues (M. Kluckow 1996) who found 27% of infants to have normal BP with low cardiac output and 25% of infants to have a normal cardiac output with low BP in 67 preterm infants with a median gestation of 28 weeks.

A negative correlation between BP and cardiac output is likely to occur if high systemic vascular resistance impedes systemic blood flow (Groves et al. 2008). A lack of positive correlation between BP and cardiac output could be explained by a few other reasons in addition to the above described ones. Left ventricular output was measured close to 20 hours of age when it is unlikely to be influenced by the left to right shunting effects of the patent ductus arteriosus (Benitz et al. 2015) and at 77 hours when the effects of ductal shunting may occur. This could lead to increased pulmonary venous return and stroke volume leading to an elevated left ventricular output. Secondly, as majority of infants had a patent ductus arteriosus at the time of scanning, peripheral vascular resistance would

also be low thus affecting mean arterial BP. Further analysis was carried out including only infants who had a haemodynamically insignificant PDA (closed or ductal diameter < 1.5 mm). Despite exclusion of these infants, no relationship was found between BP and cardiac output which means other factors described in the previous paragraph may account for a lack of direct relationship . Another reason would be the single measurements of the left ventricular output which are less informative than serial measurements. The multi-factorial nature and the inter-play between several factors described previously that occur during the transitional circulation is a potential explanation for the lack of relationship between the various physiological variables described.

3.2.5 Cerebral blood flow and blood pressure

BP (mixture of invasive and non–invasive) was significantly related to RCCA flow on day 1 postnatal life. Invasive mean arterial BP was found to be significantly related to RCCA flow on day 1 and day 3 of postnatal life. This relation persisted after only including infants who had invasive arterial BP monitoring and those who had a haemodynamically insignificant PDA (either closed or PDA with a ductal diameter of less than 1.5 mm). A hemodynamically significant PDA is associated with reduced systemic vascular resistance. Hence excluding these infants made this effect more stronger. We found that RCCA was more strongly related after excluding infants with a hemodynamically significant PDA.

There is a paucity of studies that have used Doppler ultrasound to measure cerebral blood flow volumes, hence comparison of our results is difficult. However, other markers for cerebral blood flow like SVC flow have been used. Evans et al (Kluckow and Evans 2000*a*) examined 126 infants with a mean gestation and birth weight of 27 weeks and 950

grams respectively. They found SVC flow to be correlated to BP (r=0.21, p=0.023) during the 5-hour scan only and concluded that low flow states are poorly predicted by BP. Other studies have investigated cerebral blood flow measured using NIRS and found various relationships with mean arterial BP. Some of these studies found no relation between cerebral blood flow measured using NIRS and BP (Tyszczuk et al. 1998, Meek et al. 1998, Noone et al. 2003) but others have found a direct relation with BP (Munro et al. 2004). It is debatable as to whether an increase in cardiac output or BP would increase blood flow to vital organs including the brain (Barrington 1995). However in the neonate, cerebral blood flow velocity is more dependent on BP than on the left ventricular output (Braun et al. 1986, Miall-Allen et al. 1987) and therefore BP would be a more important factor than cardiac output at least as far as cerebral circulation is concerned.

3.3 Relationship between circulatory parameters and blood gas parameters

The aim of this work was to assess the relationship between various blood gas parameters (measured as close as possible to circulatory measurements) and circulatory parameters such as cerebral blood flow and cardiac output.

Patient characteristics

All infants participating in this study underwent measurement of blood gas parameters. Physiological parameters were measured at median (IQR) age of 18 (13 –22) hours and 74 (67 –79) hours on day 1 and 3 respectively. There was a significant difference in pH, serum lactate, urine output, RCCAF and cardiac output between day 1 and day 3 of postnatal age (Table 3.2).

Clinical parameters	Day 1	Day 3	p value*
pH	7.35 (7.30 - 7.41)	7.30 (7.27 - 7.35)	**0.012**
$PaCO_2$ (KPa)	5.2 (1.5)	5.6 (1.1)	0.101
Serum lactate (mmol/L)	2.2 (1.8 - 3.5)	1.8 (1.4 - 2.1)	**<0.001**
Capillary refill time (seconds)	2 (2 - 3)	2 (2 - 2)	0.095
Urine output (ml/kg/min)	3.2 (1.7)	4.4 (1.8)	**<0.001**
RCCAF (ml/kg/min)	12.0 (9.2 - 14.8)	14.0 (11.7 - 18.0)	**0.001**
Cardiac output (ml/kg/min)	170.5 (47.2)	214.3 (69.1)	**<0.001**

Table 3.2: Table showing the circulatory parameters on day 1 and 3. Figures are expressed as mean (SD) and median (IQR). *Paired t-test for normally distributed data and Wilcoxon test for skewed data

3.3.1 Cardiac output and markers of peripheral perfusion

None of the relationships between cardiac output (left ventricular output) and the commonly used markers of peripheral perfusion achieved statistical significance. The direction of association for the non–significant relationships were complex. Cardiac output was not correlated with capillary refill time (Figure 3.15) on both days. There was no correlation between cardiac output with serum lactate (Figure 3.16) and urine output (Figure 3.17) on both days.

Cardiac output showed no correlation with renal parameters such as serum potassium (Day 1, Pearson's r = -0.008, p = 0.951), (Day 3, Pearson's r = 0.235, p = 0.073) and serum creatinine (Day 1, Pearson's r = 0.053, p = 0.688) and (Day 3, Pearson's r = -0.023, p = 0.861). None of these relationships achieved statistical significance. The correlation between cardiac output and the various markers of peripheral perfusion is summarised in table 3.23.

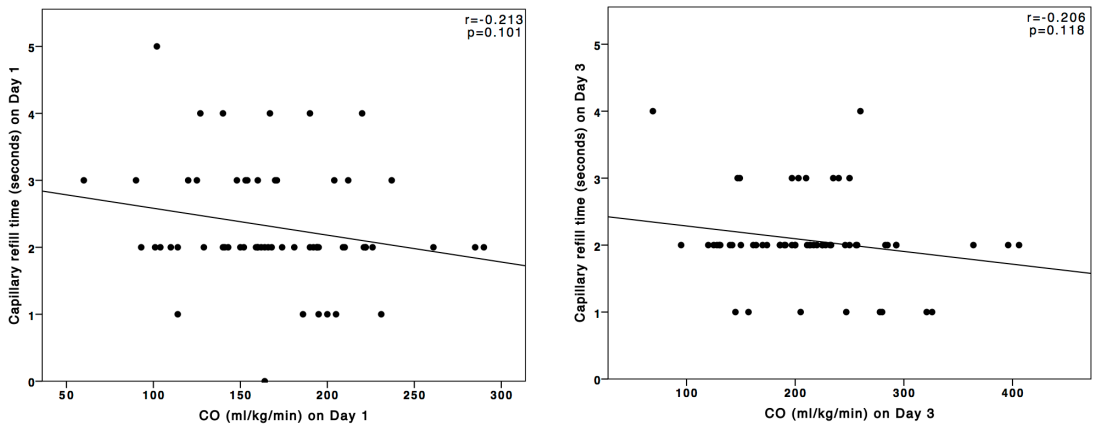


Figure 3.15: Relation between cardiac output and capillary refill time on day 1 and 3 of postnatal life. Pearson's correlation used.

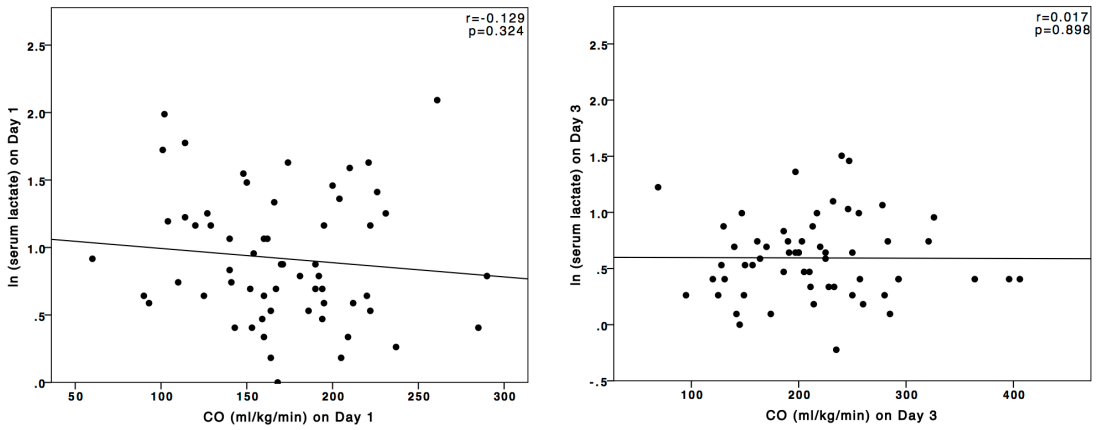


Figure 3.16: Relation between cardiac output and serum lactate on day 1 and 3 of postnatal life. Spearman's rho used.

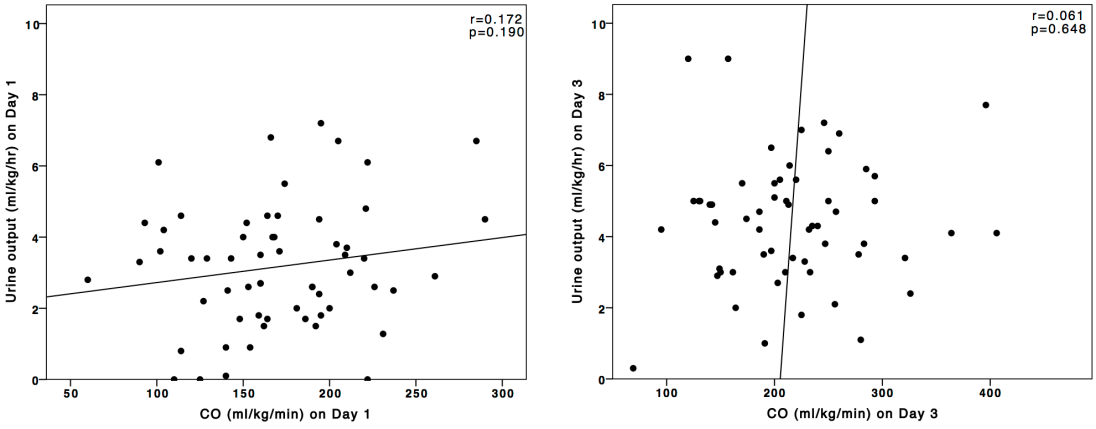


Figure 3.17: Relation between cardiac output and urine output on day 1 and 3 of postnatal life. Pearson's correlation used.

3.3.2 Cerebral blood flow and blood gas parameters

The relationship between cerebral blood flow and $PaCO_2$ (Figure 3.18), pH (Figure 3.19) and serum lactate (Figure 3.20) did not achieve statistical significance. All correlations were performed using Spearman's rho. RCCA blood flow had no relationship between pH, $PaCO_2$ and serum lactate on both days.

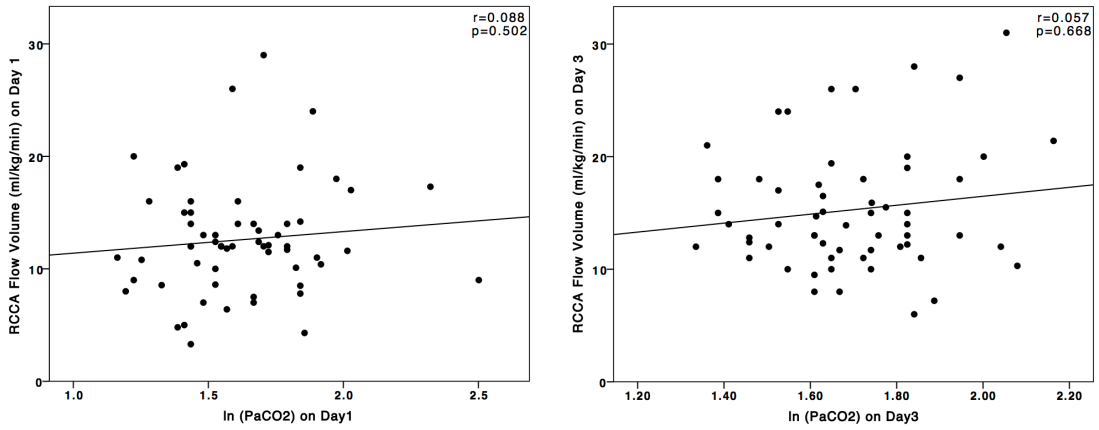


Figure 3.18: Relation between RCCA flow volume and $PaCO_2$ on day 1 and 3 of post-natal life.

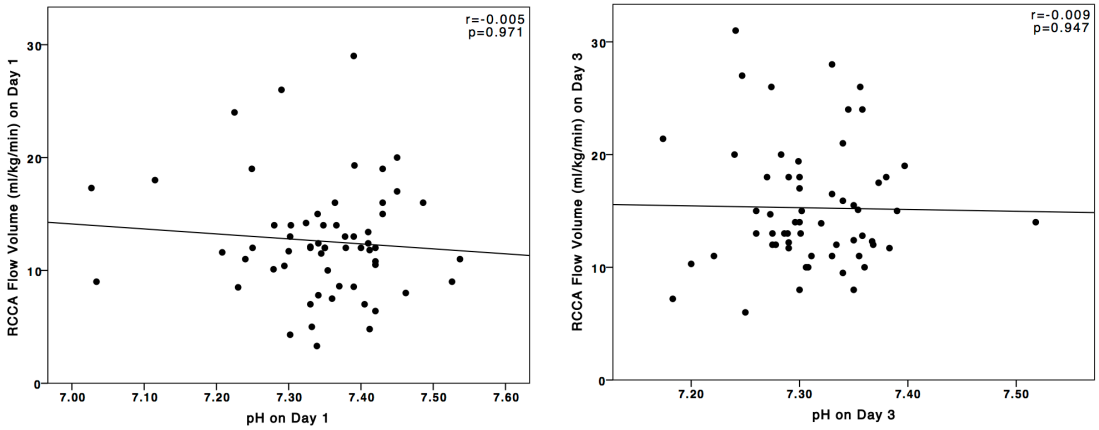


Figure 3.19: Relation between RCCA flow volume and pH on day 1 and 3 of postnatal life.

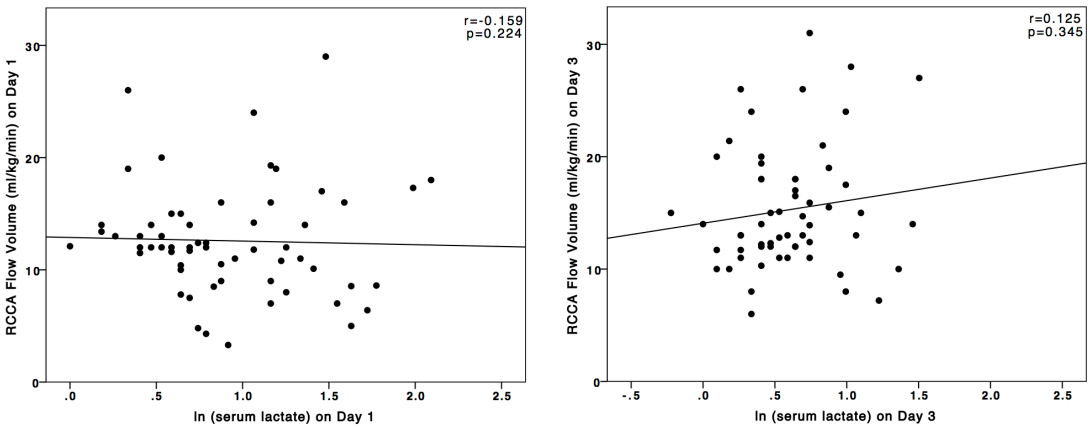


Figure 3.20: Relation between RCCA flow volume and serum lactate on day 1 and 3 of postnatal life.

Multiple regression analysis was carried out using RCCA flow volumes as the dependent variable and gestation, pCO_2, invasive BP, cardiac output and median discontinuity as the independent variables (Table 3.3). On day 1, RCCA flow volumes were predicted by invasive BP and gestational age after adjusting for pCO_2, cardiac output and median discontinuity. On day 3, we found that RCCA flow volumes were not predicted by any of the above mentioned variables in this cohort.

	Day 1 (n=50)				Day 3 (n=36)			
Clinical parameters	**Unstandardized B coefficients**	**(95%CI)**	**p value**	**Tolerance (VIF)**	**Unstandardized B coefficients**	**(95%CI)**	**p value**	**Tolerance (VIF)**
Full model								
GA	-0.67	(-1.9, 0.54)	0.27	0.70 (1.43)	1.37	(-0.67, 3.41)	0.179	0.60 (1.67)
$PaCO_2$	-0.15	(-1.1, 0.83)	0.75	0.88 (1.14)	1.15	(-0.84, 3.2)	0.246	0.81 (1.24)
Mean arterial BP	0.43	(0.12, 0.74)	**0.007**	0.81 (1.23)	0.26	(-0.27, 0.79)	0.322	0.55 (1.81)
LVO	0.01	(-0.01, 0.04)	0.30	0.91 (1.1)	0.09	(-0.03, 0.05)	0.633	0.64 (1.56)
Median discontinuity	0.09	(-0.05, 0.23)	0.22	0.64 (1.57)	0.005	(-0.17, 0.18)	0.96	0.46 (2.14)
Stepwise condensed model after elimination of non–significant variables								
GA	-1.1	(-2.1, -0.02)	**0.046**	0.9 (1.1)	1.39	(-0.15, 2.9)	0.076	1.0 (1.0)
Mean arterial BP	0.39	(0.09, 0.68)	**0.010**	0.90 (1.11)	-	-	-	-

Table 3.3: Stepwise backward multiple regression analysis between RCCA flow and clinical parameters on day 1 and 3.

3.4 Relationship between circulatory parameters and blood gas parameters - discussion

3.4.1 Cerebral blood flow and blood gas parameters

Cerebral blood flow had a non-significant relationship to $PaCO_2$ levels. Gestation and invasive BP were found to be significantly related to cerebral blood flow after adjusting for cardiac output, $PaCO_2$ and median discontinuity on day 1. None of the clinical parameters were found to be significant on day 3. Other studies (Pryds et al. 1990, Fenton et al. 1992, Levene et al. 1988) have demonstrated a direct relation between these variables. Levene and colleagues (Levene et al. 1988) who used Doppler ultrasound to measure the cerebral blood flow velocity in 19 infants (< 33 weeks gestation) found that for every increase in $PaCO_2$, there was median increase in cerebral blood flow by 44% in infants < 24 hours of postnatal age and 53% in those who were > 24 hours of postnatal age. The effect of hypercapnoea was mediated through a rise hydrogen ions, which meant that with more H^+ ions,

there was a rise in cerebral blood flow. This was caused by rising H^+ causing increased K^+ outflow from the smooth muscle cells of the cerebral arterioles thereby causing relaxation of the arterioles leading to vasodilatation (Vutskits 2014). Noori and colleagues (Noori et al. 2014) who studied $PaCO_2$ and cerebral blood flow velocity (surrogate of cerebral blood flow) in infants <30 weeks identified a breakpoint in $PaCO_2$ of around 7 KPa, meaning that cerebral blood flow reactivity was only present when the $PaCO_2$ was >7 KPa. In this study, only 5 infants had $PaCO_2$ >7 KPa which could explain the lack of relationship between RCCAF and $PaCO_2$ in this cohort. Other possible reasons include the effects of transitional circulation where one would expect clinical parameters to have more fluctuations with infants adapting to extrauterine environment and achieved homeostasis on day 3. A further possible reason could be that single RCCA blood volume measurement may not have captured the true relation between the two parameters.

3.4.2 Relationship between circulatory parameter and markers of peripheral perfusion

Cardiac output and markers of peripheral perfusion

The relationship between cardiac output and commonly used markers of peripheral perfusion were non–significant and complex. Capillary refill time had a weak negative correlation with cardiac output on both day 1 and 3, unlike other studies (Osborn 2004, Miletin et al. 2009, Wodey et al. 1998). Osborn and colleagues (Osborn 2004) found that a CRT $\geq$3 had a 55% sensitivity and 81% specificity for detecting low flow states. The lack of a significant correlation between capillary refill time and left ventricular output found in this study could be explained by several reasons. Firstly, the use of inotropes (received by the majority of infants) could result in peripheral vasoconstriction and influence car-

diac output. Secondly, cardiac output measured using the left ventricular output could be over-estimated by the presence of fetal channels. Thirdly, peripheral vasodilatation due to poor vasomotor tone in extremely preterm infants and lastly the limited measurement of physiological variables may mask the true relationship between these variables.

Serum lactate was lower on day 3 when compared to day 1, a reflection of improved systemic flow. We found a negative correlation between serum lactate and cardiac output on day 1 only with no relation on day 3. This is similar to work done by Miletin and colleagues (Miletin et al. 2009) who reported low flow states to be associated with a combination of serum lactate > 4 mmol/L and capillary refill time of > 4 seconds.

Urinary output significantly increased from day 1 to day 3 which indicates improved glomerular filtration rate secondary to increased systemic flow to the kidneys. We found cardiac output to have a non–significant positive correlation to urine output on day 1 and 3.

3.5 Relationship between blood pressure and electroencephalographic continuity in extremely preterm infants

The extremely preterm infant is at risk of increased mortality and morbidity as a consequence of multiple factors, including circulatory instability, the days immediately after birth (Kluckow and Evans 2000*a*, Sinha et al. 2006, Faust et al. 2015). Circulatory instability in the form of low BP and low flow states has been associated with adverse neurodevelopmental outcomes in this group of infants (Fanaroff and Fanaroff 2006). Despite several years of cardiovascular research, the optimum management of BP in this group of

infants remains elusive and a wide variation in practice exists (Stranak et al. 2014).

Electrocortical activity may be affected by changes in cerebral perfusion in extremely preterm infants especially more so in the first few days of life during transitional circulation (West et al. 2006, Greisen and Pryds 1989, Shah et al. 2013). The changes in electrocortical activity could be as a result of change in cerebral flow or be the cause of change in cerebral blood flow. Studies have examined several physiological parameters investigating the relationship between BP, left ventricular output and electrocortical activity (Victor, Appleton, Beirne, Marson and Weindling 2006, Victor, Marson, Appleton, Beirne and Weindling 2006). Though these studies have examined various aspects, these have been limited by patient numbers and quality of BP data. There is a paucity of good quality studies investigating the relationship between continuously measured invasive BP, cerebral blood flow and electrocortical activity in extremely preterm infants.

We investigated possible associations of pH, $PaCO_2$, lactate, morphine administration and cardiovascular measures (cerebral perfusion and continuously measured invasive mean arterial BP) with electroencephalographic measures of discontinuity in the first three days in extremely premature newborn infants.

Patient characteristics

Of the total of 111 cases assessed for eligibility, 59 were recruited to the study. 51 infants had invasive BP monitoring on day 1 and 41 infants on day 3 of postnatal life (Figure 3.21). The median (IQR) gestational age and birth weight were 25.6 (24.6–26.7) weeks and 772 (670–880) grams respectively. The patient and clinical characteristics of the infants recruited to the study are shown in table 3.4. Twenty–six infants (51%) were males. The median (IQR) pH and BE were 7.28 (7.18 –7.37) and –5.5 (–9.3, –3.2) mEq/L respec-

tively. The majority of the infants were born vaginally with median (IQR) Apgar scores of 5 (3–6) at 1 minute and 7 (6 –9) at 5 minutes of life. Thirteen (25%) infants had Grade I-II IVH and three (6%) infants had Grade III-IV IVH in the first week of life. One infant (2%) died at less than 24 hours of age and seven (14%) infants died after 24 hours of age.

The median (IQR) age of day 1 and 3 scans were 18 (13 –22) hours and 74 (67 –79) hours respectively. Sedation using morphine was administered in 16 infants on day 1 and 17 infants on day 3 of postnatal life. One infant received anticonvulsants in the first 72 hours in view of suspected clinical seizures was excluded.

EEG/aEEG

Analysable aEEG trace was obtained from 51 (100%) infants on day 1 and 36 (88%) infants on day 3 of postnatal life (Figure 3.21). Infants with poor quality EEG signal and high impedance on day 3 of life were not included for analysis as was an infant who received phenobarbitone. From day 1 to day 3, aEEG voltage increased and discontinuity decreased (Table 3.4). There was a statistically significant increase in the maximum and minimum amplitudes, a reduction in the proportion of time the minimum amplitude was below 5 microvolts and a reduction in discontinuity, from day 1 to day 3. All the EEG/aEEG parameters were significantly related to gestation and birth weight on day 1 of postnatal life (Table 3.5), with higher voltage, more continuous aEEG at higher gestation (Figure 3.22) and birthweight. Mean discontinuity is significantly related to gestational age ($p=0.004$, ANOVA).

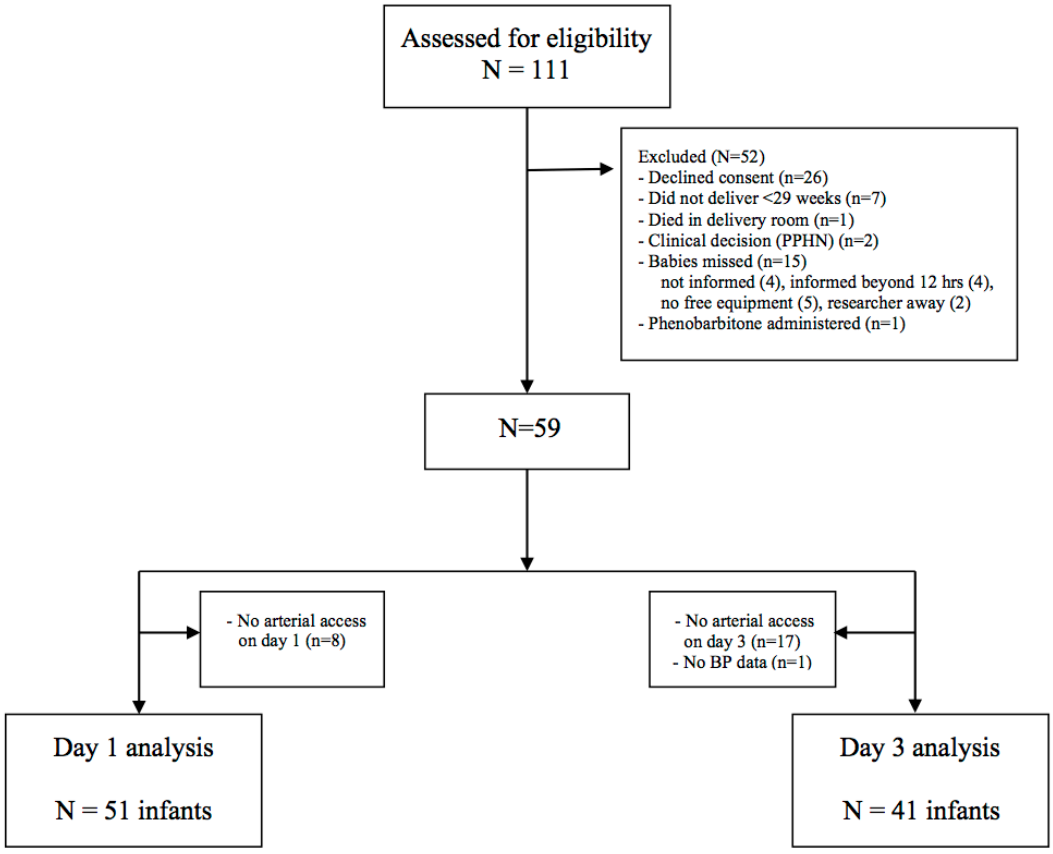


Figure 3.21: Patient flow diagram

Patient characteristics	**n=51**		
Gestational age in weeks	25.6 (24.6–26.7)		
Birth weight in grams	760 (670–880)		
Males: Females n (%)	26 (51%): 25 (49%)		
Mode of delivery, n (%)			
Vaginal delivery	46 (88%)		
Caesarean section	6 (12%)		
Cord gases[#]			
pH	7.28 (7.18–7.37)		
Base excess in mEq/l	-5.5 (-9.3, -3.2)		
Apgar score			
1 min	5 (3–6)		
5 min	7 (6–9)		
Cranial USS findings in the first 72 hours, n (%)			
Grade I or II	13 (25%)		
Grade III or IV	3 (6%)		
Death, n (%)			
< 24 hours	1 (2%)		
> 24 hours	7 (14%)		
Clinical characteristics	**Day 1** **n = 40**	**Day 3** **n = 40**	**Day 1 vs 3** **p value**
pH	7.34 (7.29 –7.41)	7.31 (7.27 –7.35)	0.34
$PaCO_2$ in mmol/L	5 (4.2–6.3)	5.3 (5–6.2)	0.47
Lactate in mmol/L	2.2 (1.7–3.5)	1.9 (1.5 –2.4)	**0.002**
Mean arterial BP in mmHg	32 (28 –35)	33 (30 –36)	0.07
LVO in ml/kg/min	170 (153 –204)	217 (186–250)	**<0.001**
RCCAF in ml/kg/min	11 (9 –14)	14 (12 –18)	**0.003**
Max aEEG amplitude (median μV)	10 (9–14)	14 (9 –18)	**< 0.001**
Min aEEG amplitude (median μV)	3 (2–4)	4 (3 –4)	**< 0.001**
% Min amplitude < 5 μV	96 (82 –99)	80 (59 –95)	**< 0.001**
Mean discontinuity in seconds	25 (19 –31)	17 (9–25)	**0.012**
Median discontinuity in seconds	24 (17 –31)	15 (7–26)	**0.008**

Table 3.4: Patient and clinical characteristics. Where not specified, all figures are expressed as median (interquartile range). [#] n=14. Continuous variables were compared for infants who had invasive arterial lines on both day 1 and 3 only. Paired t–test or Chi–squared test used

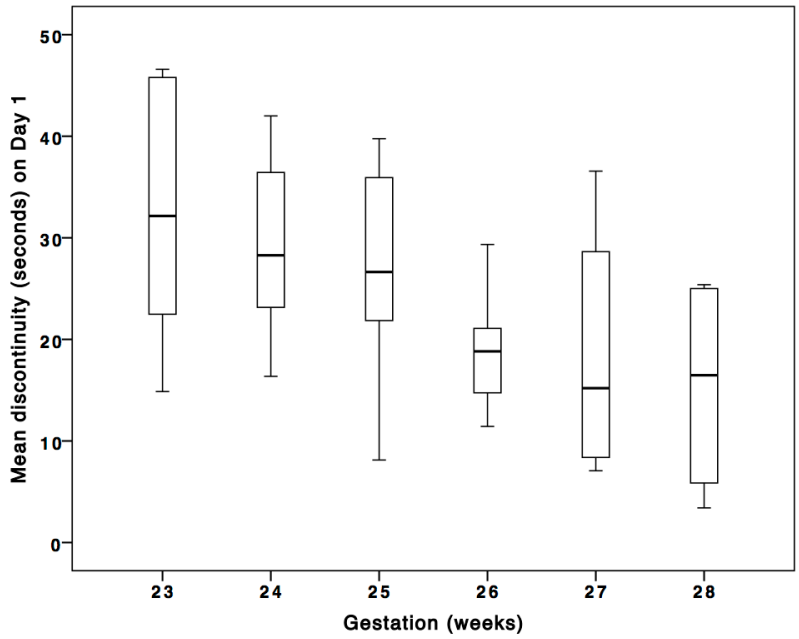


Figure 3.22: Box plots illustrating the effect of gestation on mean discontinuity on day 1 of postnatal life. Solid lines within boxes represent median values, edge of boxes representing 1st and 3rd quartile and whiskers representing minimum and maximum values.

Discontinuity or periods of electrical quiescence predominated the electroencephalogram in the very immature infant. This is in contrast to the more mature infant where electrocortical activity in the form of delta waves occurred more frequently representing cortical maturity. This period of electrical quiescence reduced with increasing gestational age as shown below (Figures 3.23, 3.24, 3.25).

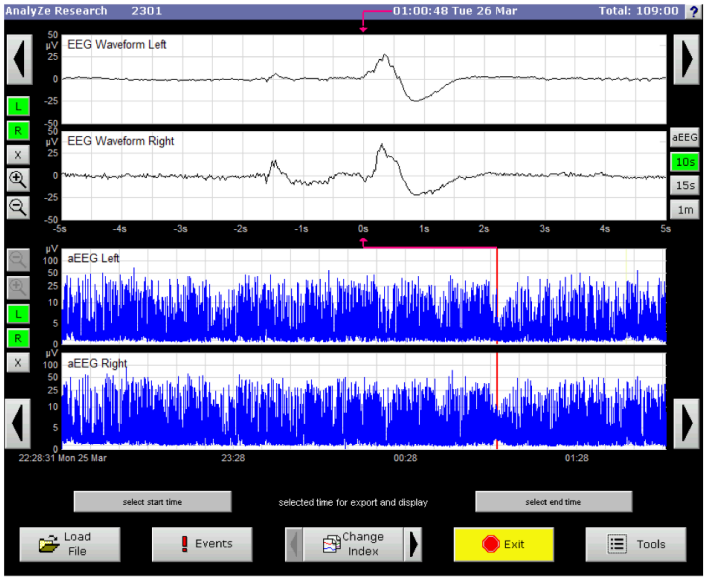


Figure 3.23: Raw EEG and aEEG trace in a infant born at 23 weeks gestation.

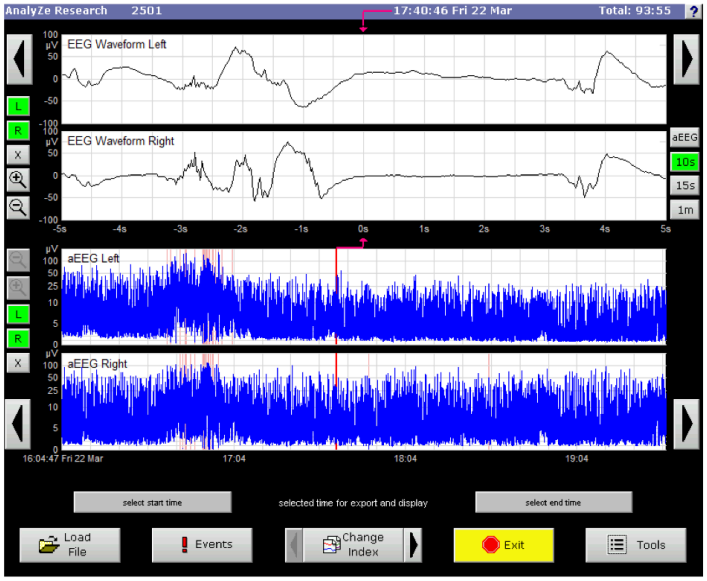


Figure 3.24: Raw EEG and aEEG trace in a infant born at 25 weeks gestation.

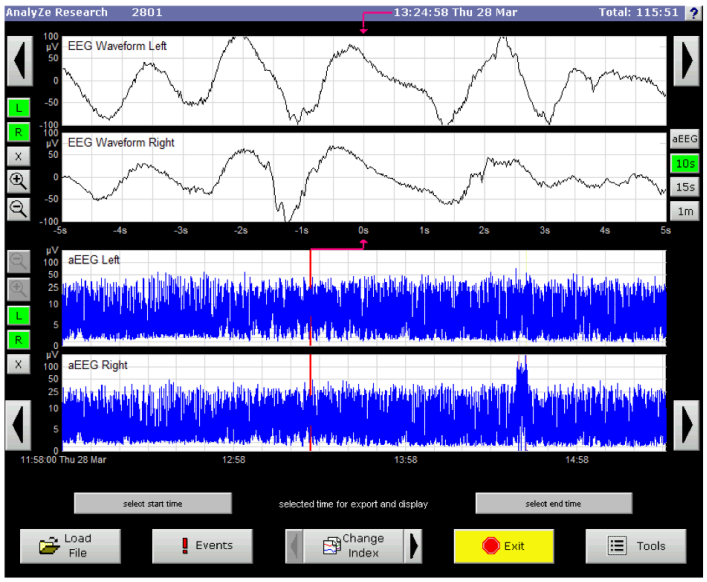


Figure 3.25: Raw EEG and aEEG trace in a infant born at 28 weeks gestation.

Morphine administration significantly ($p < 0.001$) suppressed all aEEG parameters. For those not receiving morphine, vs those on morphine, median (IQR) values were: maximum amplitude 13 (10-16) vs 8 (6-10) microvolts, minimum amplitude 3.5 (2.6-4) vs 2.3 (2-2.7) microvolts, discontinuity 20 (13-25) vs 36 (27-41) seconds (Figures 3.26, 3.27, 3.28). Morphine administration also produced similar significant changes in all aEEG parameters on day 3 of postnatal life. For those not receiving morphine, vs those on morphine, median (IQR) values were: maximum amplitude 16 (13-19) vs 10 (7-14) microvolts ($p=0.002$), minimum amplitude 3.9 (3.4-4.6) vs 2.4 (2-3.7) microvolts ($p=0.003$), discontinuity 13 (9-21) vs 23 (12-40) seconds ($p=0.022$).

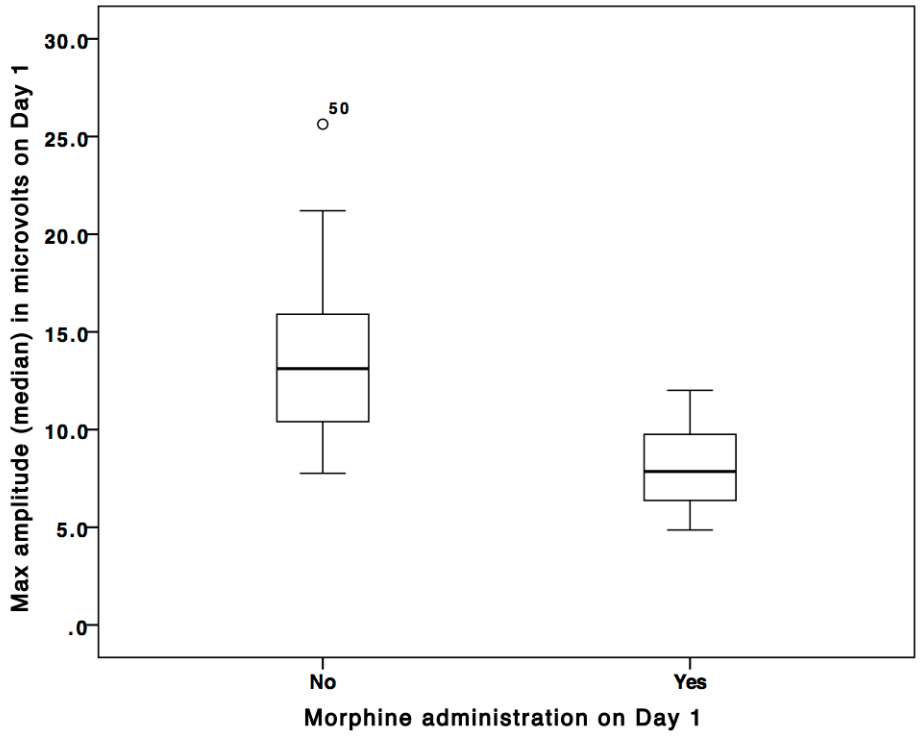


Figure 3.26: Box plot illustrating the effect of morphine on maximum EEG amplitude on day 1 of postnatal life. ($p<0.001$)

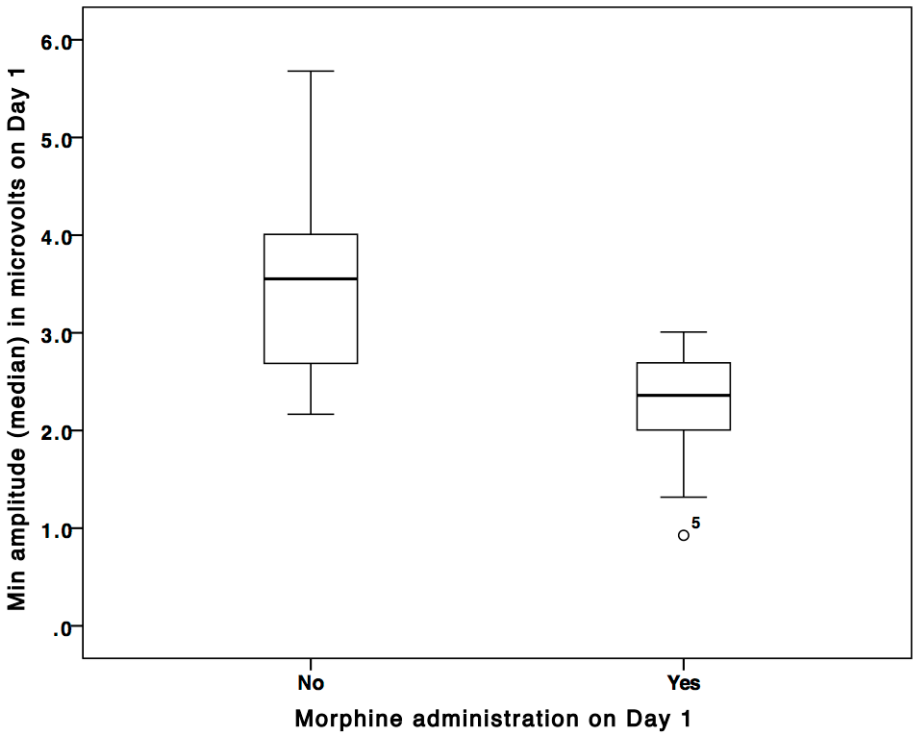


Figure 3.27: Box plot illustrating the effect of morphine on minimum EEG amplitude on day 1 of postnatal life. ($p<0.001$)

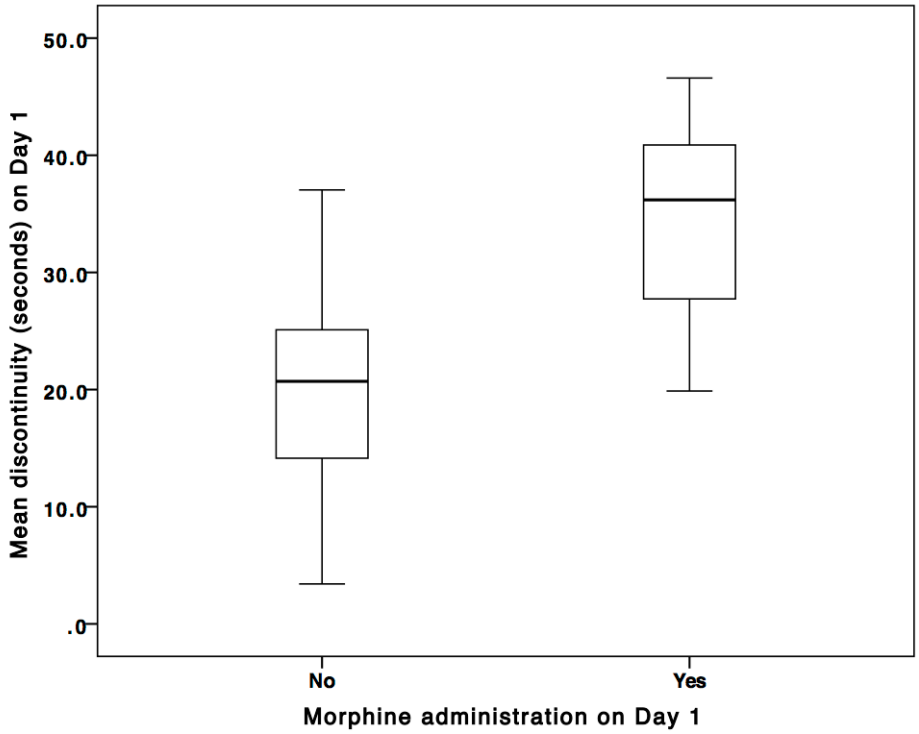


Figure 3.28: Box plot illustrating the effect of morphine on discontinuity on day 1 of postnatal life. ($p<0.001$)

The majority of infants received dopamine as first line inotropic treatment with only three infants receiving dopamine and dobutamine. Infants receiving inotropes had significantly ($p < 0.001$) suppressed aEEG parameters on day 1 of postnatal life only. For those not receiving inotropes, vs those on inotropes, median (IQR) values were: maximum amplitude 14 (11-17) vs 9 (8-11) microvolts, minimum amplitude 3.7 (2.8-4.5) vs 2.5 (2.2-3.0) microvolts, median discontinuity 16 (12-24) vs 29 (21-37) seconds. On day 3, for those not receiving inotropes, vs those on inotropes, median (IQR) values were: maximum amplitude 14 (9-18) vs 14 (9-19) microvolts ($p=0.66$), minimum amplitude 3.6 (2.9-4.4) vs 3.5 (2.3-5.3) microvolts ($p=0.20$), median discontinuity 18 (9-24) vs 14 (10-28) seconds ($p=0.78$).

Inotrope administration was significantly correlated to maximum (Figure 3.29 A) and minimum (Figure 3.29 B) amplitude and discontinuity (Figure 3.29 C) on day 1. A increase in the dose of inotropic support was associated with a reduction in the maximum and minimum amplitude of EEG and an increase in discontinuity, both of which suggesting suppression of EEG with the use of inotropes. The correlation between inotrope dosage and aEEG parameters were less so on day 3 (Figure 3.29 D–F).

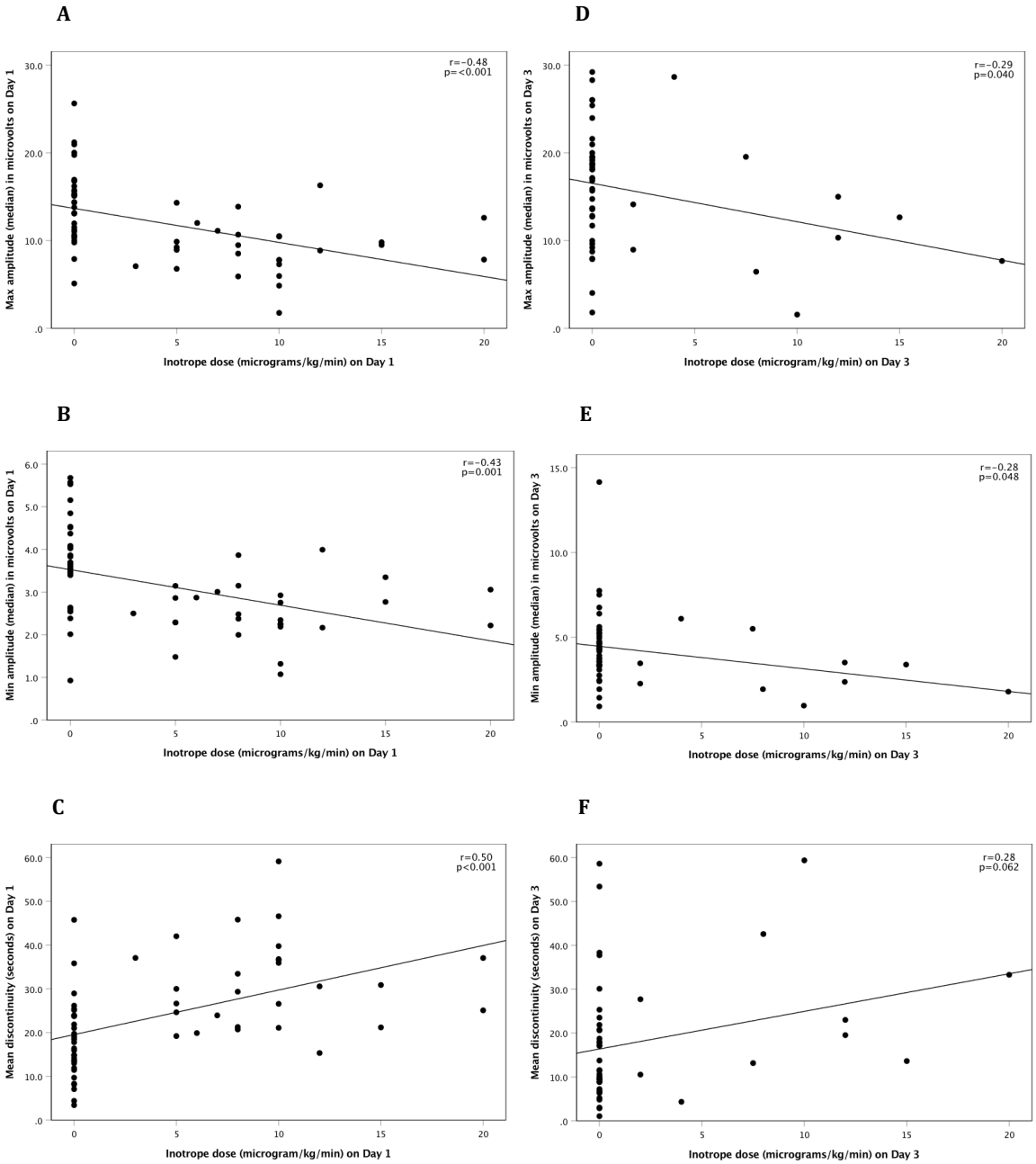


Figure 3.29: Scatter plot illustrating the effect of inotropes on maximum and minimum EEG amplitude and mean discontinuity on day 1 (A-C) and 3 (D-F) of postnatal life.

On Day 1 of postnatal life 90% of infants had a patent ductus arteriosus (PDA) and this was not associated with any difference in EEG parameters. For those infants without PDA, vs those with presence of PDA, median (IQR) values were: maximum amplitude 9 (8-15) vs 11 (8-15) microvolts (p=0.66), minimum amplitude 2.5 (1.9-4.1) vs 3.0 (2.4-3.7) microvolts (p=0.60), median discontinuity 30 (17-38) vs 24 (16-30) seconds (p=0.44). By Day 3, 74% of the infants had a PDA. There were no significant differences in EEG parame-

ters. For those infants without PDA, vs those with presence of PDA, median (IQR) values were: maximum amplitude 19 (10-20) vs 13 (9-16) microvolts (p=0.22), minimum amplitude 4.5 (2.9-5.4) vs 3.5 (2.4-4.2) microvolts (p=0.11), median discontinuity 11 (9-28) vs 19 (9-25) seconds (p=0.84).

Blood gas parameters were associated with mean discontinuity on day 1 and 3 (Table 3.5, Figures 3.30, 3.31, 3.32). Acidosis, higher carbon dioxide and lactate values, were related to lower voltage and less continuous aEEG. The effects of pH on day 3 were less consistent (Table 3.5, Figure 3.30).

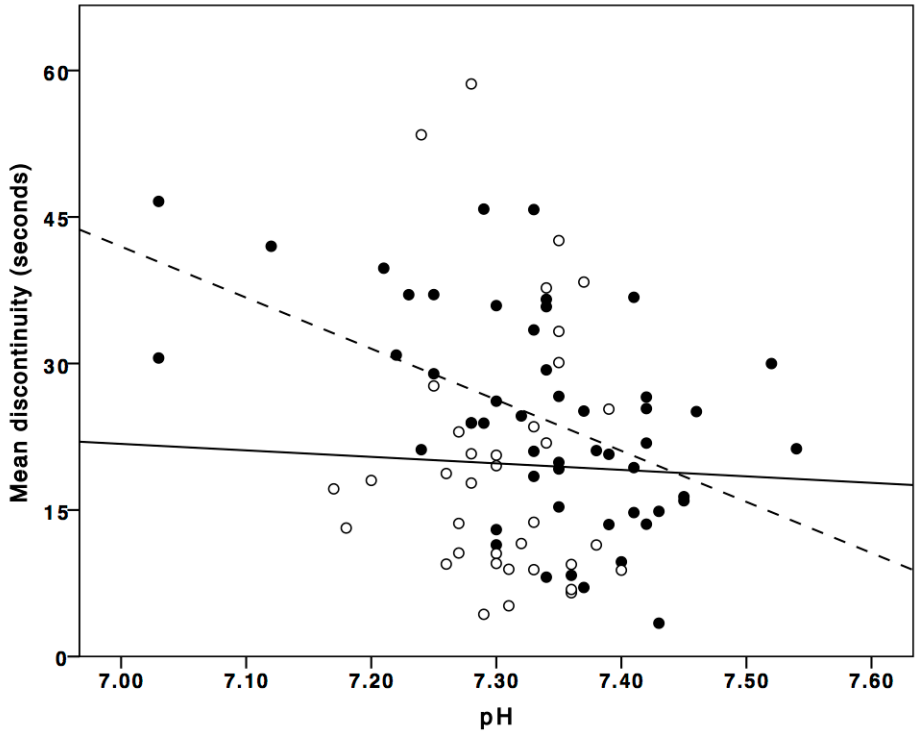


Figure 3.30: Correlation between mean discontinuity and pH. Day 1 (r=-0.495, p < 0.001) represented by closed dots and dashed line. Day 3 (r=-0.028 , p=0.872) represented by open dots and solid line. Pearson's correlation used.

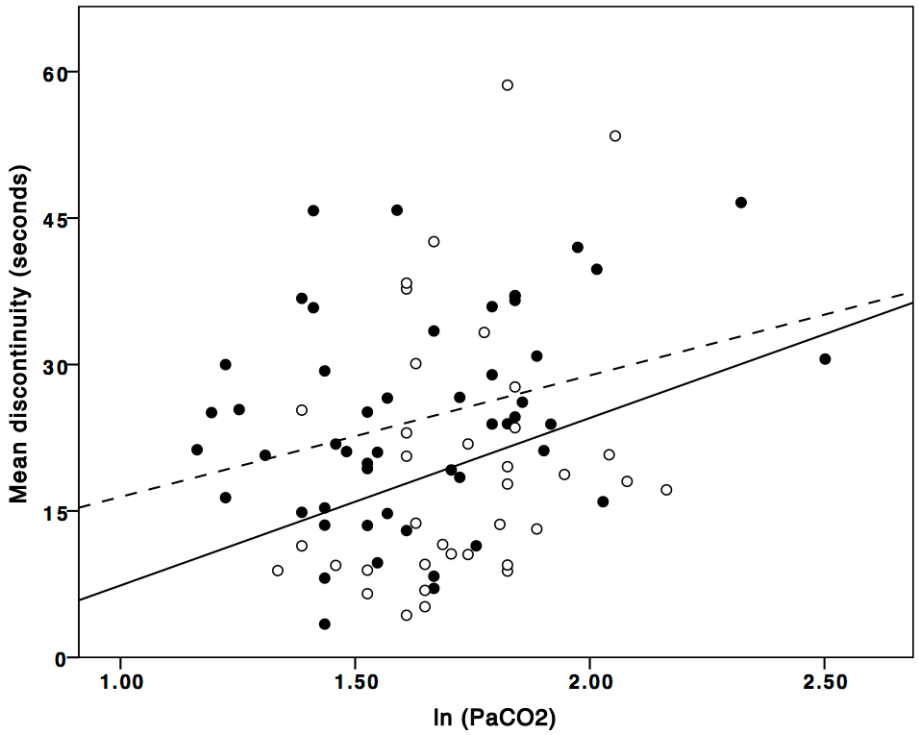


Figure 3.31: Correlation between mean discontinuity and natural log scale of $PaCO_2$. Day 1 (r=0.340, p=0.015) represented by closed dots and dashed line. Day 3 (r=0.24 , p=0.152) represented by open dots and solid line. Pearson's correlation used.

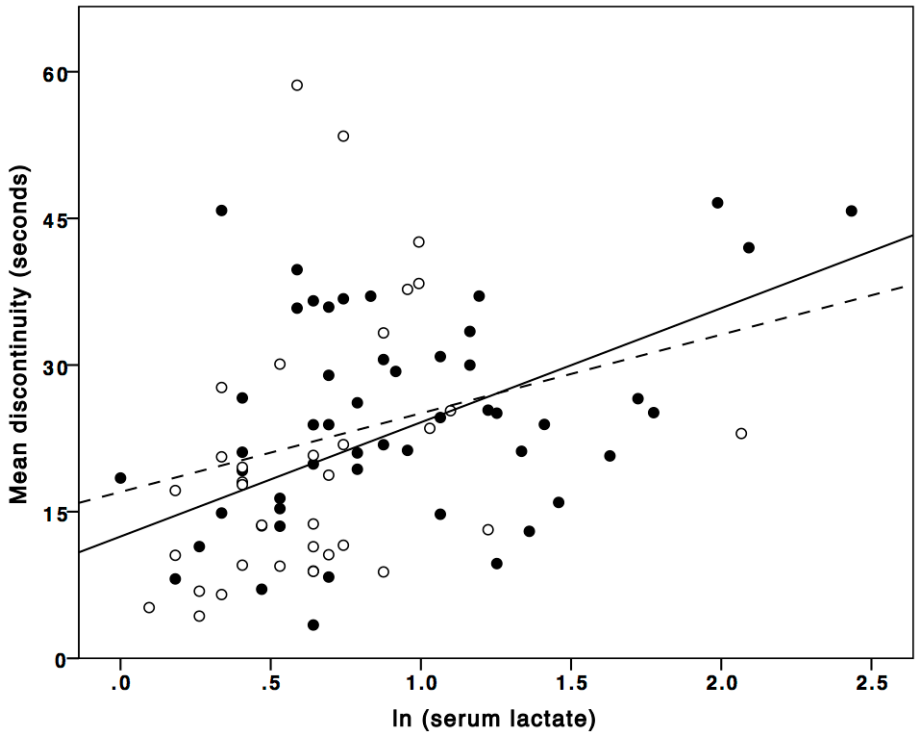


Figure 3.32: Correlation between mean discontinuity and natural log scale of serum lactate. Day 1 (r=0.432, p=0.002) represented by closed dots and dashed line. Day 3 (r=0.232 , p=0.174) represented by open dots and solid line. Pearson's correlation used.

BP was significantly correlated with aEEG/EEG measures of continuity on both day 1 and

day 3 of life (Figure 3.33, Table 3.5). There was no correlation between RCCAF and the various EEG parameters on day 1 or 3 of postnatal life.

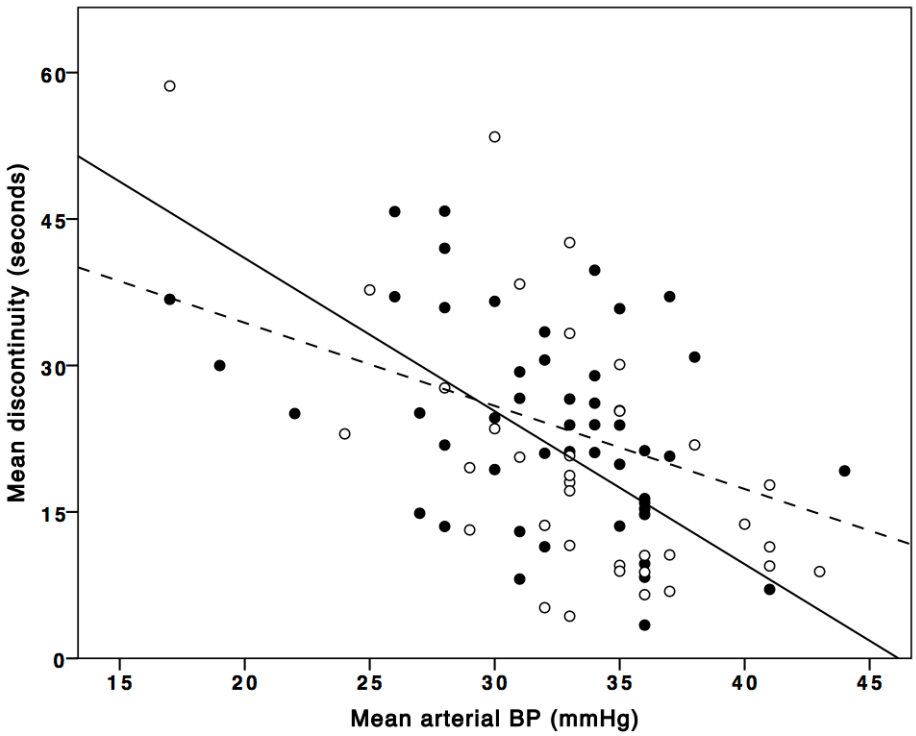


Figure 3.33: Correlation between mean discontinuity and BP. Day 1 (r=-0.414, p=0.003) represented by closed dots and dashed line. Day 3 (r=-0.612, p<0.001) represented by open dots and solid line. Pearson's correlation used.

Using stepwise multiple regression analysis (Table 3.6) we found that low BP remained significantly associated with EEG discontinuity on day 1 (β = - 0.67, p = 0.001) and day 3 (β = - 1.60, p < 0.001) of postnatal life after adjusting for gestation, pH, $PaCO_2$, lactate, RCCAF, LVO, morphine and inotrope administration. Acidosis, morphine and inotrope administration were found to be associated with EEG discontinuity on day 1 only. This relationship persisted after excluding the three infants who had grade III-IV intraventricular haemorrhage. Though invasive BP measurements are considered the gold standard, in reality not all infants may have invasive lines inserted. This could be due to malpositioning, blockage of lines, inability to insert arterial lines. We also investigated the relationship between measures of EEG/aEEG continuity with infants who had both invasive and non-

invasive mean BP to reflect what one would encounter in day to day clinical practice. We found that measures of EEG/aEEG continuity were both related to mean BP on day 1 and day 3 of postnatal life. This relationship was stronger on day 3.

Clinical Characteristics	Day 1 (n = 51)				Day 3 (n = 41)			
	Max aEEG amplitude (median µV)	Min aEEG amplitude (median µV)	% Min amplitude <5 µV	Discontinuity (mean) in sec on day 1	Max aEEG amplitude (median µV)	Min aEEG amplitude (median µV)	% Min amplitude <5 µV	Discontinuity (mean) in sec on day 3
Gestation (weeks)	0.507***	0.486***	-0.518***	-0.522***	0.086	0.082	-0.190	0.107
pH	0.434**	0.450**	-0.372**	-0.495***	0.071	0.007	-0.021	-0.028
$PaCO_2$	-0.365**	-0.327*	0.269	0.340*	-0.315	-0.268	0.286	0.224
Serum lactate in mmol/L	-0.376**	-0.455**	0.213	0.423**	-0.265	-0.267	0.272	0.232
Invasive mean arterial BP (mmHg)#	0.364*	0.408**	-0.377**	-0.414**	0.533**	0.546**	-0.461**	-0.612***
LVO (ml/kg/min)	0.199	0.093	0.015	-0.212	-0.220	-0.278	0.312	0.193
RCCAF (ml/kg/min)	0.063	0.097	-0.082	-0.002	0.073	0.093	-0.135	-0.006

Table 3.5: Relationship between aEEG parameters and various clinical characteristics. Correlation between variables examined using Pearson's correlation. (* p <0.05, ** p < 0.01, *** p < 0.001).
Matched invasive only BP and aEEG data available in 51 infants on day 1 and 36 infants on day 3.

	Day 1 (n=52)				Day 3 (n=43)			
Clinical parameters	**Unstandardized B coefficients**	**(95%CI)**	**p value**	**Tolerance (VIF)**	**Unstandardized B coefficients**	**(95%CI)**	**p value**	**Tolerance (VIF)**
Full model								
GA	-0.69	(-2.4,1.0)	0.42	0.60 (1.67)	5.28	(2.71, 7.85)	**< 0.001**	0.71 (1.41)
pH	-25.4	(-64.0, 13.3)	0.19	0.27 (3.70)	85.7	(7.79, 163.6)	**0.032**	0.42 (2.36)
$PaCO_2$	0.23	(-1.9,2.4)	0.83	0.30 (3.35)	5.45	(1.4,9.5)	**0.010**	0.38 (2.64)
Lactate	0.98	(-0.1,2.1)	0.08	0.83 (1.20)	0.78	(-2.06,3.6)	0.57	0.71 (1.41)
Mean arterial BP	-0.66	(-1.1, -0.23)	**0.003**	0.76 (1.32)	-1.53	(-2.2, -0.84)	**< 0.001**	0.60 (1.67)
Morphine	7.97	(3.0,12.9)	**0.03**	0.65 (1.53)	8.39	(1.36,15.4)	**0.021**	0.67 (1.49)
Inotropes	4.27	(-0.05,8.6)	0.053	0.74 (1.36)	-8.66	(-15.5,-1.78)	**0.016**	0.76 (1.32)
RCCAF	0.14	(-0.33,0.60)	0.55	0.76 (1.31)	-0.51	(-1.1, 0.07)	0.08	0.64 (1.56)
LVO	-0.02	(-0.06,0.02)	0.31	0.81 (1.23)	0.04	(-0.01,0.09)	0.116	0.81 (1.23)
Stepwise condensed model after elimination of non–significant variables								
GA	-	-	-	-	4.69	(2.2,7.2)	**0.001**	0.77 (1.29)
pH	-28.8	(-49.9,-7.7)	**0.009**	0.86(1.16)	83.8	(5.2,162.4)	**0.038**	0.43 (2.34)
$PaCO_2$	-	-	-	-	4.64	(0.76 ,8.52)	**0.021**	0.42 (2.37)
Mean arterial BP	-0.67	(-1.0,-0.29)	**0.001**	0.90 (1.11)	-1.60	(-2.3, -0.94)	**<0.001**	0.69 (1.45)
Morphine	8.84	(4.5,13.2)	**< 0.001**	0.79 (1.25)	9.76	(2.85,16.7)	**0.007**	0.71 (1.41)
Inotropes	4.87	(0.9,8.85)	**0.017**	0.82 (1.22)	-8.86	(-15.6, -2.1)	**0.012**	0.80 (1.25)
LVO	-	-	-	-	0.09	(0.03,0.15)	**0.003**	0.89 (1.12)

Table 3.6: Stepwise backward multiple regression analysis between mean discontinuity and clinical parameters on day 1 and 3

A causal relationship between BP and EEG could be missed with the above 'aggregate'

approach. In order to make the statistical testing more robust and to address between and within subject variance and shortcomings associated with a simple approach as above, with assistance from a statistician, we ran further testing using a mixed effects model. Using mixed effects multiple regression analysis, factors that influenced mean EEG discontinuity include gestation ($\beta = 3.57$, $p=0.001$), $PaCO_2$ ($\beta = 9.48$, $p=0.009$), serum lactate ($\beta = 4.24$, $p=0.028$), morphine ($\beta = 9.85$, $p<0.001$) and invasive mean arterial BP ($\beta = -1.04$, $p<0.001$). LVO and RCCAF were not associated with any EEG discontinuity (Table 3.7).

Clinical parameters	B coefficients	Standard error	(95% CI)	p - value
GA	3.57	1.04	(1.5, 5.6)	**0.001**
$PaCO_2$	9.48	3.65	(2.3, 16.6)	**0.009**
Lactate	4.24	1.93	(0.5, 8.0)	**0.028**
Morphine	9.85	1.89	(6.1, 13.6)	**< 0.001**
Mean arterial BP	-1.04	0.18	(-1.4, -0.7)	**< 0.001**

Table 3.7: Mixed effects multiple regression analysis between mean EEG discontinuity and clinical parameters.

3.6 Relationship between blood pressure and electroencephalographic continuity in extremely preterm infants - discussion

3.6.1 aEEG/EEG parameters

On day 1 of postnatal life, all aEEG/EEG parameters measured in this study were significantly related to gestational age consistent with previous reports (Vesoulis et al. 2014, Zhang et al. 2011, Olischar et al. 2004, Hayakawa et al. 2001, Vecchierini et al. 2007, Hellstrom-Westas et al. 2006, Selton 2000), such that with increasing gestational age, the maximum amplitude, minimum amplitude, percentage of time minimum amplitude is < 5 microvolts and discontinuity increased. The values of median discontinuity on day 1

(21 seconds versus 15 seconds) and day 3 (9 seconds versus 9 seconds) of postnatal life were comparable to other studies examining infants of a similar gestation (Victor, Marson, Appleton, Beirne and Weindling 2006, Victor et al. 2005*b*). All aEEG/EEG parameters examined showed a significant difference between day 1 and day 3 of postnatal life suggesting increasing electrocortical activity in the first few days.

3.6.2 Electroencephalographic parameters and blood gas parameters

We found acidosis and hypercarbia to have a suppressive effect on most aEEG parameters on day 1 of life but not on day 3 as previously reported (Granot et al. 2012, Wikstrom et al. 2011, Victor et al. 2005*a*, Eaton et al. 1994*b*). The suppression of EEG caused by hypercarbia may be exerted through changes in pH. Our work demonstrated pH having a suppressive effect on EEG. Hypercarbia results in decreased energy metabolism (characterised by lower adenosine triphosphate and phosphocreatinine levels), altered neuronal enzyme activity and protein expression in piglet models (Fritz et al. 2005). The lack of relation between $PaCO_2$ and EEG on day 3 may be related to cerebral haemodynamics as described by Victor and associates (Victor et al. 2005*a*). We know that cerebral oxygen delivery increases in the first few days after birth characterised by a reduction in cerebral fractional oxygen extraction (Kissack et al. 2004), increase in cardiac output (Evans and Kluckow 1996, Sirc et al. 2013), systemic BP (Cunningham et al. 1999) and cerebral blood flow (Meek et al. 1998). Further work (Levene et al. 1988, Fenton et al. 1992, Pryds et al. 1989) has shown that cerebral vasoreactivity to changing $PaCO_2$ increases with postnatal age. It is plausible that on day 1 of postnatal life, when cerebral perfusion is at its lowest, the effect of $PaCO_2$ on EEG was most marked and subsequently on day 3 of postnatal life, with increasing cerebral perfusion (supported by a significant reduction in serum lactate on day 3 in our cohort) despite cerebral vasoreactivity, cerebral electrical activity was pre-

served.

3.6.3 Electroencephalographic parameters and physiological variables

There were no consistent significant association between EEG/aEEG measures of continuity and blood flow parameters similar to other studies (Shah et al. 2013, Victor, Appleton, Beirne, Marson and Weindling 2006). We found that RCCA blood flow volumes were not related to any of the aEEG parameters on day 1 and 3 of life. There are a few possible reasons for this lack of association. One possibility is that the relationship between systemic BP and measures of EEG/aEEG continuity are not simply mediated by alterations in systemic blood flow transmitted to the cerebral circulation. Secondly, cerebral auto regulation could play a role and has been shown to be inconsistently related to BP (Popat et al. 2013) in this group of infants. Thirdly, the frequency of RCCA flow measurement, performed once in the majority of infants on day 1 and 3 of life, would not have captured these changes. Fourthly, cardiac output may be over-estimated due to the presence of ductus arteriosus shunting and lastly, BP may not fall until the natural compensatory mechanisms finally fail but serious reductions in cardiac output may be detected earlier. (Victor, Marson, Appleton, Beirne and Weindling 2006). The median cardiac output measures were comparable to previously published data from hypotensive preterm neonates, but slightly lower than in more mature babies with higher BP and gestation (M. Kluckow 1996).

3.6.4 Electroencephalographic parameters and blood pressure

We found a strong correlation between invasive mean arterial BP and various measures of EEG/aEEG activity (such as maximum and minimum EEG margin, percentage of time EEG was < 5 microvolts and discontinuity) in extremely preterm neonates in the first three

days of life. We also found that this association between invasive mean arterial BP and various measures of EEG continuity persisted after adjusting for common clinical characteristics (pH, $PaCO_2$, serum lactate, common carotid artery blood flow, left ventricular output, morphine and inotrope administration) which could influence the preterm EEG during the two time points on day 1 and 3 of postnatal life. EEG measures of continuity remained associated with invasive mean arterial BP after excluding infants who had grade III-IV intraventricular haemorrhage.

In contrast to other studies examining this area, all infants in this study had the gold standard invasive arterial BP data which was continuously extracted every 10 seconds for the first week of life. Our findings were replicated by other studies. West et al (West et al. 2006) showed that BP at 12 and 24 hours of age was related to aEEG continuity at the same time epoch. They also found that infants in the lowest quartile for BP, which was 31 mmHg, had lower aEEG continuity. Further work by Victor et al (Victor, Marson, Appleton, Beirne and Weindling 2006) concluded that infants with mean BP above 30 mmHg were found to have normal EEG continuity. There are several possible mechanisms by which EEG continuity could decrease at lower BP levels, before reductions in cerebral perfusion affect cellular energy status. This could be postulated to be part of an intrinsic cerebral protective response to hypotension, with lower electrocortical activity reducing neuronal oxygen demand. In response to hypoxia-ischaemia, a neuroprotective adenosine mediated suppression of EEG has been studied in animal models (Ilie et al. 2006, Hunter et al. 2003).

3.6.5 Effect of medication on electroencephalographic parameters

The suppressive effect of morphine administration on all aEEG parameters is consistent with previous reports in neonates (Norman et al. 2013, Young and da Silva 2000). In these studies, morphine administration was associated with prolonged depression of the EEG and a reduction in the BP. However, electrocortical depression occurred independent of the BP. The suppression seen with inotropes on aEEG parameters found on day 1 is most likely to be due to prior hypotension triggering inotropic support, as there was no effect seen on Day 3 once BP levels had stabilised.

3.7 Blood pressure measurements

Invasive and non–invasive BP measurements were recorded in this study. These were continuously downloaded from the monitor every 10 seconds for the first week of life. In addition to this, staff recorded invasive and non–invasive BP measurements recorded in the case report forms were also studied. In the following pages, I will compare the 'gold standard' invasive BP measurements first followed by a mixture of invasive and non–invasive BP measurements followed by staff recorded BP measurements. BP from different sources will be analysed using 4–hourly intervals and then at 24–hourly intervals for the first week of life.

3.7.1 Continuously downloaded invasive blood pressure

Invasive BP measurements were available for 51 (85%) infants between 8 and 72 hours of age. The median (range) age for recruitment of infants into the study was 8.1 (1–12) hours. The maximum number of infants fell into this time bracket because before 8 hours infants were still being recruited into the study and after 72 hours, majority of infants had invasive arterial lines removed as they were more stable. Before 8 hours and after 72

hours, there was a significant drop in the number of infants in each arm.

A) 4 –hourly invasive blood pressure

4 –hourly invasive BP measurements were available in 17 (89%) infants in the Active arm, 18 (90%) infants in the Moderate arm and in 16 (76%) infants in the Permissive arm. There was a significant difference in BP between 12 to 15 hours and 16 to 19 hours among the three arms of the study as illustrated in the table 3.8 and figure 3.34

	Active N=19	**Moderate N=20**	**Permissive N=21**	**p value**
Number of patients with data analysed	17 (89%)	18 (90%)	16 (76%)	
Average MABP 8-11 hours (N 16,16,13)	31.7 (30.8-34.2)	32.3 (29.9-35)	30 (28.4-33.3)	0.128
Average MABP 12-15 hours (N 17,17,16)	33.4 (31.1-36.4)	30.3 (28-33.6)	27.4 (24.9-30.2)	**<0.001**
Average MABP 16-19 hours (N 17,18,16)	34.1 (31.2-37.6)	32 (28.1-33.9)	30.4 (26.8-33)	**0.007**
Average MABP 20-23 hours (N 17,18,16)	35.1 (31.9-37.1)	32.1 (30.2-35.5)	31.7 (29.9-35.5)	0.120
Average MABP 24-27 hours (N 17,18,16)	34.2 (32.1-35.2)	34.4 (31.3-40)	34.3 (32.2-38)	0.822
Average MABP 28-31 hours (N 17,18,16)	33.1 (30.9-35.9)	35.7 (29.3-38.6)	34.6 (30.8-37.4)	0.604
Average MABP 32-35 hours (N 17,18,15)	34 (31.2-37.7)	36.1 (31.8-39.7)	33.2 (30.4-35.5)	0.539
Average MABP 36-39 hours (N 17,17,15)	34.6 (32.2-37)	35.1 (33.2-40.1)	31.5 (29.9-36)	0.177
Average MABP 40-43 hours (N 17,17,15)	34.8 (33.6-37.6)	35.1 (33.2-37.9)	31.9 (29.4-35.4)	0.068
Average MABP 44-47 hours (N 17,17,15)	34.5 (33-38.6)	34.9 (32.8-38.2)	33.2 (29.3-35.1)	0.183
Average MABP 48-51 hours (N 14,13,13)	35.0 (31.6-36.7)	32.6 (31.8-36.2)	32.2 (28.8-35.9)	0.095
Average MABP 52-55 hours (N 14,13,13)	34.0 (32.4-35.2)	34.7 (31.3-36.8)	31.9 (28.9-37.0)	0.384
Average MABP 56-59 hours (N 14,13,13)	34.1 (32.2-36.1)	34.0 (32.0-36.9)	32.3 (28.2-37.4)	0.332
Average MABP 60-63 hours (N 14,13,13)	35.6 (33.8-37.1)	33.3 (28.2-37.9)	31.6 (27.3-37.0)	0.115
Average MABP 64-67 hours (N 14,13,13)	35.2 (33.1-37.2)	34.3 (30.3-38.7)	31.3 (27.9-37.2)	0.123
Average MABP 68-71 hours (N 14,13,13)	34.1 (33.7-36.7)	34.3 (30.9-38.4)	32.5 (26.4-38.1)	0.176

Table 3.8: Table illustrating 4–hourly invasive BP measurements between 8 to 72 hours of life. Values are median (IQR).

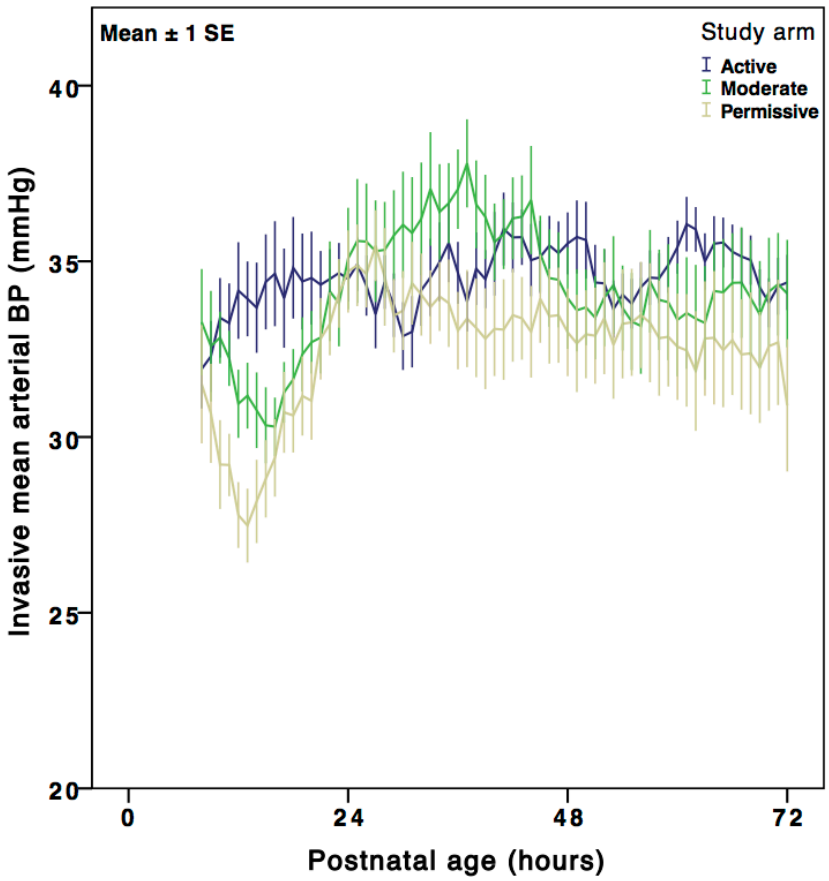


Figure 3.34: Invasive mean arterial BP in the three arms of the study during the first 3 days of life

B) 24–hourly invasive blood pressure

24–hourly invasive BP measurements were available in 17 (89%) infants in the Active arm, 18 (90%) infants in the Moderate arm and in 16 (76%) infants in the Permissive arm as illustrated in table 3.9. As shown in the table below, the majority of infants had invasive BP data unto 72 hours of age. After 72 hours, there was a significant drop in the number of infants with invasive BP data. This reduction in invasive BP data represents the majority of those infants who were stable (both cardiovascularly and clinically), in whom the clinicians felt did not require invasive arterial lines any longer. The trend of BP for the three arms of the study is shown in figure 3.35. A rise in BP in the three arms of the study was noted on day 5 to day 6 of postnatal age.

	Active N=19	Moderate N=20	Permissive N=21	p value
Number of patients with data analysed	17 (89%)	18 (90%)	16 (76%)	
Average MABP 1-24 hours (N 17,18,16)	33.1 (31.6-34.9)	30.9 (29.2-34.8)	29.7 (28.3-32.4)	**0.008**
Average MABP 25-48 hours (N 17,18,16)	34 (32.9-36.1)	35.5 (32.9-39.2)	33.4 (31.1-36.1)	0.545
Average MABP 49-72 hours (N 16,13,14)	34.1 (33.5-36.5)	33.6 (31.6-37.5)	32 (27.4-37.3)	0.086
Average MABP 73-96 hours (N 13,13,9)	34.2 (31-35.6)	33.1 30.8-36.2)	31.9 (26.6-36.8)	0.370
Average MABP 97-120 hours (N 11,10,8)	49 (45.5-53)	46.1 (44.3-52.6)	47 (36.7-54.2)	0.400
Average MABP 121-144 hours (N 10,8,7)	50.1 (46.8-53)	47 (43.2-51.2)	45.6 7.5-54.7)	0.342
Average MABP 145-168 hours (N 9,8,6)	34.1 (31-39.1)	33.6 (27.7-36.4)	31.5 (27.6-33.6)	0.089

Table 3.9: 24–hourly invasive BP measurements for the first week of life. Values are median (IQR).

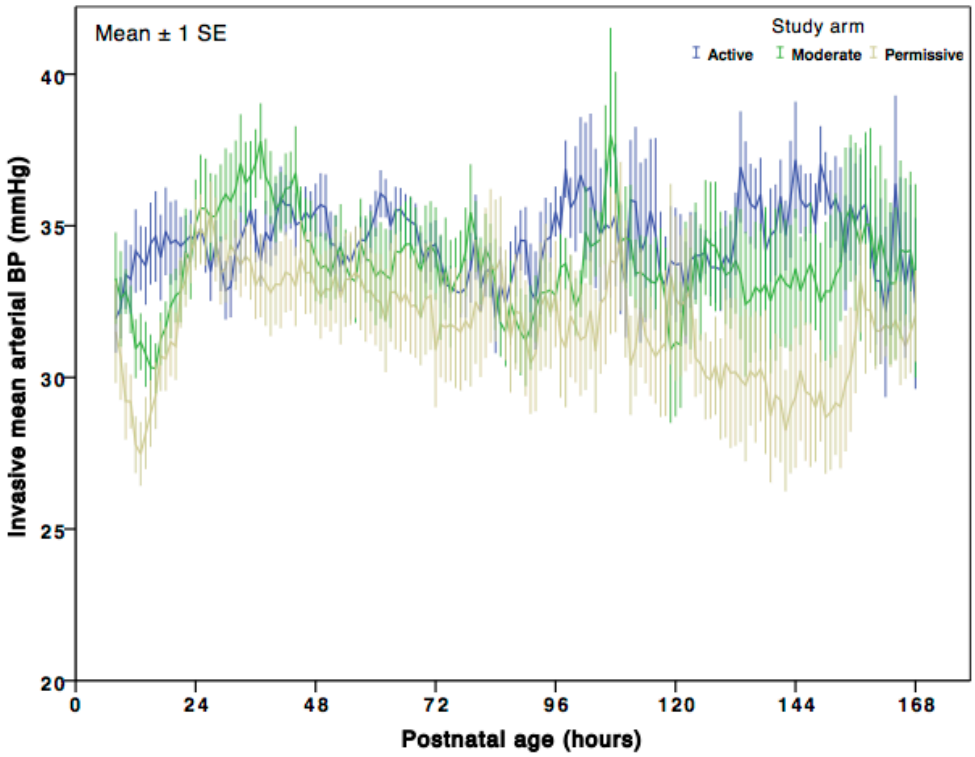


Figure 3.35: Invasive mean arterial BP in the three arms of the study during the first week of life

Further statistical analysis was carried out using different methods of comparing BP between the three arms of the study after recommendation from a statistician was sought. The mean BP for each of the three arms were compared to the entire groups overall mean BP. This method of analysis used data from the entire cohort and analysed to see if there was a significant difference during any particular time period. The graph shown below (Figure 3.36) highlights the periods in blue where the mean BP was significantly different from the entire group.

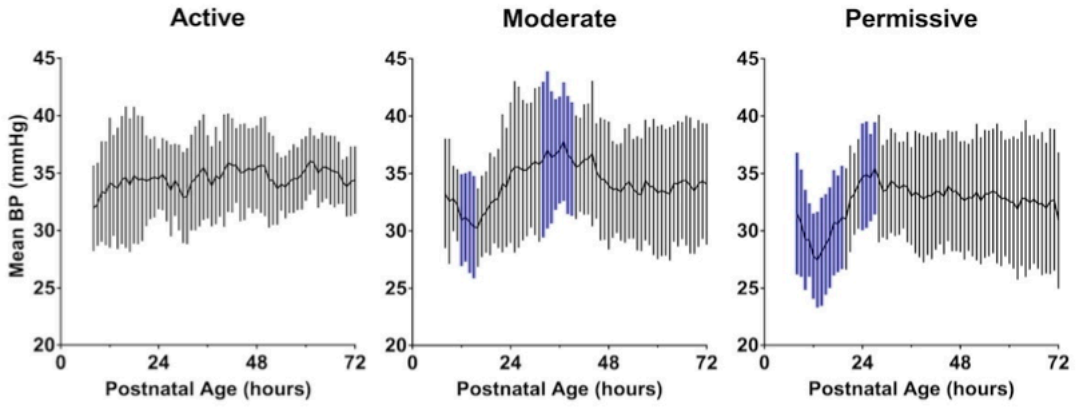


Figure 3.36: Mean Arterial BP in each study arm. Periods when BP was significantly different from the overall average are indicated by blue/dark bars. (Values are hourly mean +/-SD, for patients with invasively measured BP; Active=17, Moderate=18, Permissive=16. Statistical analysis by mixed effects GLM, with Benjamini-Hochberg correction).
Image courtsey –Dr. Steve Kempley

As invasive BP is considered the 'gold standard', further detailed analysis of BP data was restricted to the 51 infants (85%) who had continuous invasive BP monitoring. Similar numbers were included for this invasive BP analysis from each study arm (Active n=17, Moderate n=18, Permissive n=16); most of those without invasive arterial lines were more mature in the 27-28 week gestation range. On day 3 of postnatal age, infants requiring invasive respiratory support was significantly lower in the 27-28 week group when compared to the 23-24 week group (38% versus 100%, p=0.006, Fisher's exact test). As clinicians were more likely to insert arterial lines in infants receiving invasive ventilation,

the more mature group had more non–invasive BP measurements, mimicking what one would expect to encounter in clinical practice.

On the first postnatal day, invasive BP was highest in the Active arm. BP remained the most stable in this arm when compared to other arms, with no significant differences from the overall mean BP for all groups in the first 3 days of life (Figure 3.36). In the Moderate arm, BP was significantly reduced from the overall mean at 12-15 hours of age (-3.1mmHg, 95% CI -5.2 to -1.0), and became significantly elevated at 32-39 hours of age (+3.5mmHg at 36-39 hrs, 95% CI 1.3 to 5.6). In the Permissive arm, BP was lowest on day 1 compared to the other two arms. BP was significantly reduced at 8-19 hours of age (-5.2mmHg at 12-15 hrs, 95% CI -6.3 to -4.1), with a short period of significant elevation at 24-27 hours (+1.7mmHg, 95% CI 0.6 to 2.7). After 72 hours, there were no major differences in BP between the groups (Table 3.9).

The dispersion of BP data between the three arms for the first 72 hours was different (Figure 3.37). The mean SD (SD) were 3.8 (0.9) mmHg in the Active arm, 6 (1.4) mmHg in the Moderate arm and 7 (1.4) mmHg in the Permissive arm which were significantly different ($p<0.001$, ANOVA). In the Active arm, there was a downward trend in the SD beyond 24 hours of age when compared to the Moderate and Permissive arm, both of which, had an upward trend in SD after 24 hours. Infants in the Active arm had the lowest SD in BP contrasting with infants in the Permissive arm who had the highest SD.

The trend of SD for the first week of life (Figure 3.38) demonstrated a notable increase in the SD in all three arms of the study after the first 72 hours postnatal age. This is consistent with an increase BP that is known to occur beyond the first three days of postnatal age.

The mean SD (SD) for BP in the first week of life was significantly different between the Active (6.2 (2.7) mmHg), Moderate (8.6 (2.9) mmHg) and Permissive (9.4 (2.5) mmHg) arms ($p<0.001$, ANOVA). On Day 7, the SD was highest in the Moderate arm followed by the Active and the Permissive arm. The number of infants in each arm of the study was highest between 12 to 48 hours postnatal age in the first week of life.

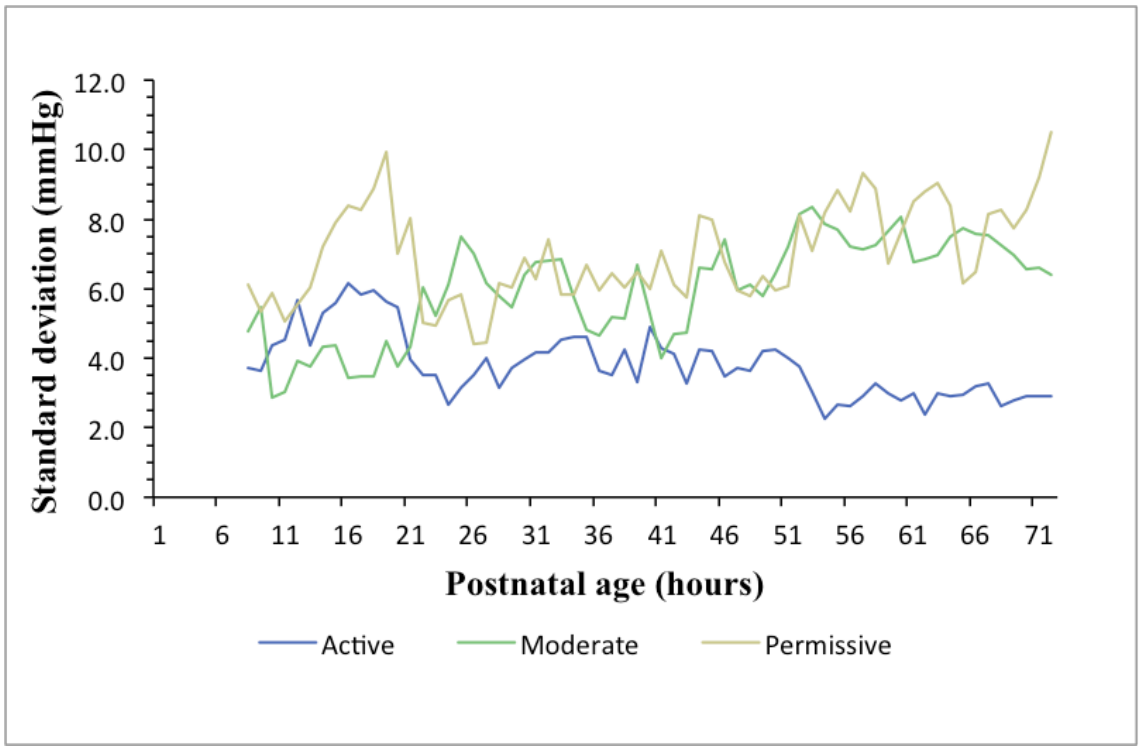


Figure 3.37: Standard deviation for BP in the three arms of the study in the first 72 hours.

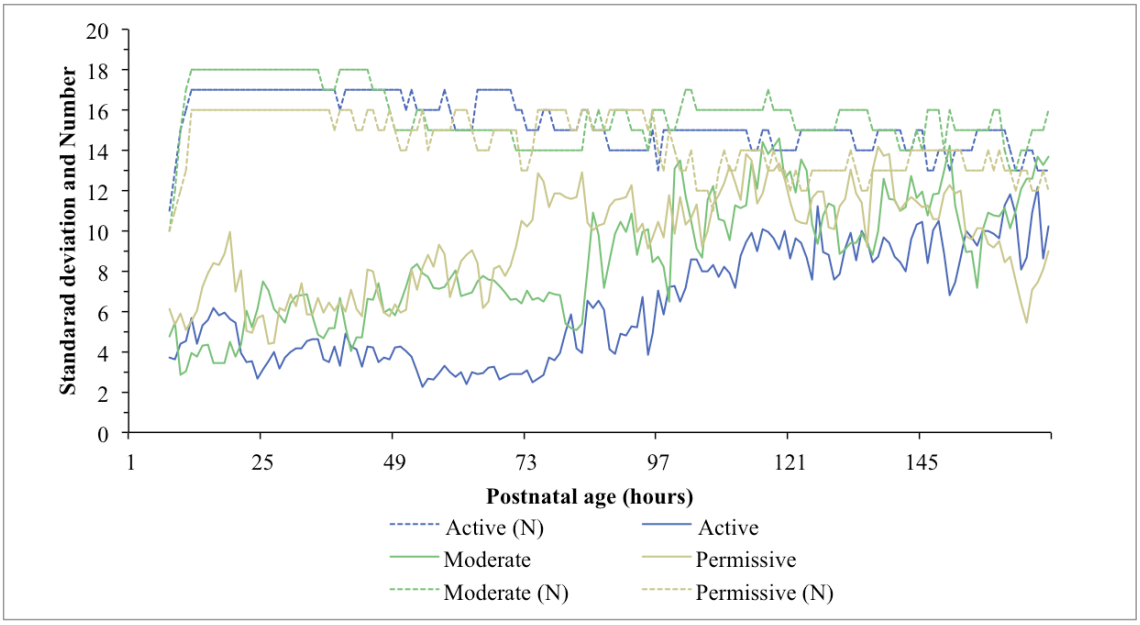


Figure 3.38: Standard deviation (solid lines) for BP and number of infants (dotted lines) in the three arms of the study for the first week of life.

3.7.2 Continuously downloaded invasive blood pressure and non-invasive blood pressure

In the first 72 hours, invasive BP measurements were available for 51 (85%) infants and non–invasive BP for 9 (15%) infants due to reasons described earlier. In view of the majority of infants having invasive BP, analysing infants who only had non-invasive BP data will result in very small numbers between the three arms and would not give meaningful results. Therefore analysis was performed using a mixture of invasive and non-invasive BP data.

A) 4–hourly invasive and non-invasive blood pressure

There was a significant difference between the three arms of the study between 12–15 hours of age, with the BP being highest in the Active and lowest in the Permissive arm of the study (Table 3.10) with no other significant difference between the three arms of the study for the first week of life (Figure 3.39). The Active arm had the highest BP in the first 24 hours, preserving the order of randomisation. The Moderate arm had the lowest

and highest BP at about 3 and 5 days respectively. This is in contrast to invasive only BP measurements, where the order of randomisation was preserved during these time periods.

	Active N=19	**Moderate N=20**	**Permissive N=21**	**p value**
Continuously downloaded invasive and non-invasive blood pressure				
Number of patients with data analysed	17 (89%)	18 (90%)	16 (76%)	
Average MABP 8-11 hours (N 18,17,17)	32.6 (30.8-34.3)	32.1 (30.1-35.0)	30.8 (28.9-35.8)	0.551
Average MABP 12-15 hours (N 19,18,21)	35.7 (31.2-36.8)	30.4 (28-33.4)	28.4 (25.8-37.8)	**0.012**
Average MABP 16-19 hours (N 19,18,21)	34.6 (31.9-37.7)	32 (28.1-3.9)	31.4 (27.9-39.4)	0.143
Average MABP 20-23 hours (N 19,18,21)	35.1 (32.1-38.7)	32.1 (30.2-35.5)	34.5 (30.1-39.7)	0.553
Average MABP 24-27 hours (N 19,18,21)	34.5 (32.5-38)	34.4 (31.2-40)	35.2 (32.8-41.5)	0.335
Average MABP 28-31 hours (N 19,18,21)	33.2 (31-36.8)	35.7 (29.3-38.6)	35.7 (31.2-41.8)	0.206
Average MABP 32-35 hours (N 19,18,21)	34.8 (31.6-38.8)	36.1 (31.8-39.7)	35.3 (31.7-44.2)	0.544
Average MABP 36-39 hours (N 19,18,21)	35 (32.6-39.2)	35.6 (33.2-41.5)	33.4 (30.4-43.8)	0.960
Average MABP 40-43 hours (N 19,18,20)	34.8 (33.7-38.9)	35.3 (33.4-38.7)	33.2 (30.1-45.3)	0.618
Average MABP 44-47 hours (N 19,17,20)	48.3 (44.9-53.7)	46.7 (43.6-49.9)	49.2 (42.4-61.4)	0.874

Table 3.10: 4–hourly mixture of invasive and non–invasive BP measurements between 8 to 48 hours of life.Values are median (IQR).

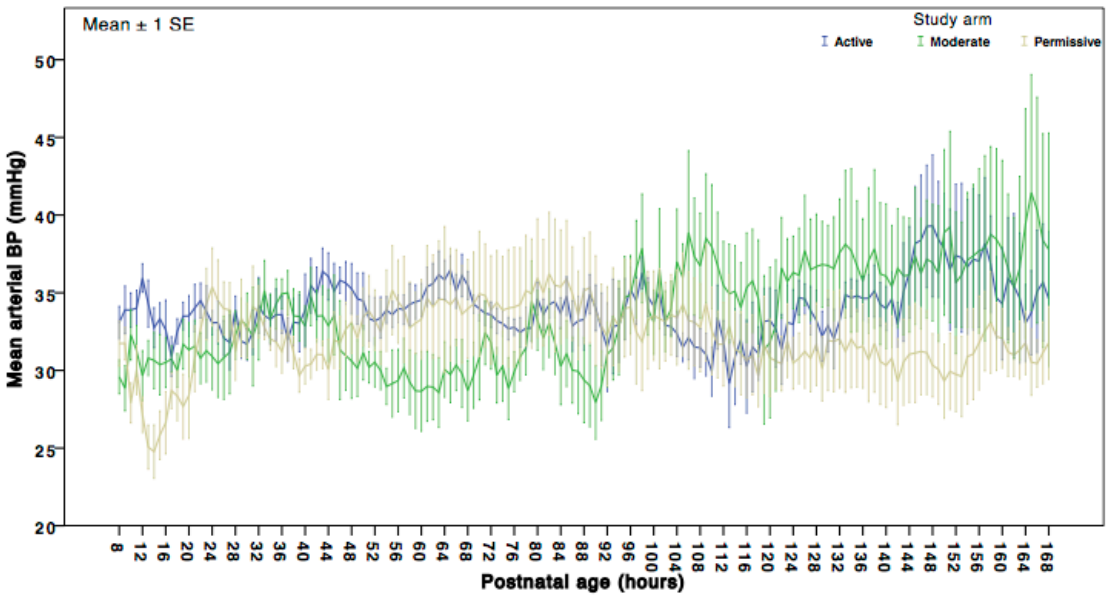


Figure 3.39: Continuously downloaded invasive and non–invasive mean arterial BP in the three arms of the study from 8 hours to 168 hours.

B) 24–hourly invasive and non-invasive blood pressure

24–hourly invasive BP measurements were available in 19 (100%) infants in the Active arm, 18 (90%) infants in the Moderate arm and in 21 (100%) infants in the Permissive arm as shown in table 3.11. Though the number of infants in each did not reduce by a large extent, there were no significant differences between the arms which could be explained due the averaging of the BP measurements.

	Active N=19	Moderate N=20	Permissive N=21	p value
Number of patients with data analysed	19 (100%)	18 (90%)	21 (100%)	
Average MABP 1-24 hours (N 19,18,21)	33.7 (31.7-35.6)	30.9 (29.7-34.8)	30.9 (28.7-39.4)	0.132
Average MABP 25-48 hours (N 19,18,21)	34.8 (33.2-37.1)	35.5 (32.9-39.2)	34.3 (31.2-44.4)	0.684
Average MABP 49-72 hours (N 19,16,21)	34.1 (33.5-37.7)	35.2 (32.4-39.6)	39.8 (31-45.5)	0.827
Average MABP 73-96 hours (N 19,17,20)	36 (33-39.1)	34.9 (31.8-41.7)	39.9 (31.1-51.7)	0.552
Average MABP 97-120 hours (N 17,17,18)	35.8 (32.2-45.1)	37.9 (32-49.9)	42.3 (32-52.9)	0.360
Average MABP 121-144 hours (N 17,17,17)	38.1 (34.4-48.3)	39.4 (32.4-50)	40.5 (31-52.6)	0.762
Average MABP 145-168 hours (N 17,16,17)	40.9 (34-46.5)	42.8 (33.5-50.8)	42.9 (32.4-50)	0.824

Table 3.11: Table illustrating 24–hourly mixture of invasive and non–invasive BP measurements for the first week of life. Values are median (IQR).

3.7.3 Staff recorded blood pressure

Staff recorded BP (mixture of invasive and non-invasive BP and invasive only BP) documented in the study case report forms were examined at different time intervals. The 4–hourly BP (Table 3.12), 24–hourly BP measurements (Table 3.13) and trend of BP during the first week of life (Figure 3.40) are shown. The only significant difference was noted when invasive BP measurements were taken into account between 12–16 hours of age. The BP trend shows that the order of randomisation is not followed here and for the majority of time periods the Permissive arm was found to have the highest BP measurements between the three arms of the study.

(A) 4–hourly staff recorded blood pressure measurements

	Active N=19	Moderate N=20	Permissive N=21	p value
Staff recorded invasive and non-invasive blood pressure				
Number of patients with data analysed	19 (100%)	20 (100%)	21 (100%)	
Average MABP 8-11 hours (N 19,20,21)	31.5 (30-34.2)	33.6 (30.8-37.3)	29.7 (27.5-37.1)	0.867
Average MABP 12-16 hours (N 19,20,21)	32.5 (30.5-36)	30.7 (28.6-34.2)	30 (26.7-39)	0.305
Average MABP 20-24 hours (N 19,20,21)	36 (33-37.5)	34.2 (29.5-39.2)	34.5 (31-38.2)	0.555
Average MABP 28-32 hours (N 19,19,21)	34 (31-37.5)	36 (31.5-38.5)	37 (33-44.7)	0.060
Average MABP 36-40 hours (N 19,19,21)	34 (33-38)	37 (34.5-40)	34.5 (31.5-44.5)	0.392
Average MABP 44-48 hours (N 19,19,21)	37 (33.5-39.5)	36 (33.5-38.5)	35 (30.7-47.2)	0.835
Staff recorded invasive blood pressure				
Number of patients with data analysed	17 (89%)	16 (80%)	16 76%)	
Average MABP 8-11 hours (N 17,16,16)	31.5 (29.6-34.2)	33.6 (30.8-38.2)	28.9 (26-34.7)	0.205
Average MABP 12-16 hours (N 17,16,16)	31.5 (30.2-34.5)	30.5 (28.6-33.5)	28.5 (26.5-32.1)	**0.039**
Average MABP 20-24 hours (N 17,16,16)	35.5 (33-37.2)	34.2 (28.7-37.9)	33.5 (30-35.4)	0.208
Average MABP 28-32 hours (N 17,16,16)	34 (30.2-37.2)	35 (31.5-38.4)	35 (32.5-37.9)	0.321
Average MABP 36-40 hours (N 17,16,16)	34 (32.5-36.2)	36.2 (34.5-39.2)	32.5 (30.6-38.9)	0.869
Average MABP 44-48 hours (N 17,16,16)	35.5 (33-39.2)	35.7 (33.1-37.4)	33.5 (29.2-35.9)	0.160

Table 3.12: 4–hourly staff recorded (mixture of invasive and non–invasive BP measurements and invasive only BP measurements) from 8 to 48 hours of life. Values are median (IQR).

(B) 24–hourly staff recorded blood pressure measurements

	Active N=19	Moderate N=20	Permissive N=21	p value
Staff recorded invasive and non-invasive blood pressure				
Average MABP 1-24 hours (N 19,20,21)	32.3 (31.3-34.9)	33 (29.5-35.6)	32.1 (28.4-39.2)	0.968
Average MABP 25-48 hours (N 19,19,21)	34.8 (32.8-37.7)	35.8 (33.3-38.8)	35.7 (32.5-44.5)	0.373
Average MABP 49-72 hours (N 19,19,21)	34 (32.7-39.3)	34.8 (32.2-39.8)	39.2 (31.3-46.5)	0.540
Average MABP 73-96 hours (N 19,19,21)	35.3 (33.3-38.3)	34.5 (30.8-45)	39.8 (31.1-51.9)	0.396
Average MABP 97-120 hours (N 19,19,21)	35 (31.5-42.5)	39.5 (33.5-49.8)	37.7 (32.4-53.2)	0.486
Average MABP 121-144 hours (N 19,19,19)	37.5 (31.7-46.5)	40.4 (32-49.2)	38.5 (31.7-54.8)	0.312
Average MABP 145-168 hours (N 19,19,20)	40.7 (33.2-45)	43.3 (32.7-48.2)	46.4 (33.4-51)	0.357
Staff recorded invasive blood pressure				
Number of patients with data analysed	17 (89%)	16 (80%)	16 76%)	
Average MABP 1-24 hours (N 17,16,16)	32.2 (31.1-34.9)	32.3 (29-35.5)	30.4 (27.8-33.3)	0.202
Average MABP 25-48 hours (N 17,16,16)	34.3 (32.3-36.4)	35.7 (33.1-38.7)	34 (31.4-36.6)	0.905
Average MABP 49-72 hours (N 17,16,16)	33.8 (32.7-36.6)	34.6 (31.9-38)	32.3 (31.2-40.9)	0.769
Average MABP 73-96 hours (N 17,16,16)	34.7 (31.7-36.7)	34.1 (30.8-41.9)	32.7 (29.9-45.7)	0.847
Average MABP 97-120 hours (N 17,16,16)	34.8 (31.4-41.6)	35.6 (32.7-48.9)	34.1 (29.6-48.8)	0.876
Average MABP 121-144 hours (N 17,16,15)	36.7 (31.1-42.7)	38.6 (31.2-47)	34.3 (30.8-51.8)	0.798
Average MABP 145-168 hours (N 17,16,16)	40.2 (33.2-42.9)	42 (32.5-47.1)	35.9 (32.4-49.6)	0.819

Table 3.13: 24–hourly staff recorded (mixture of invasive and non–invasive BP measurements and invasive only BP measurements) for first week of life. Values are median (IQR).

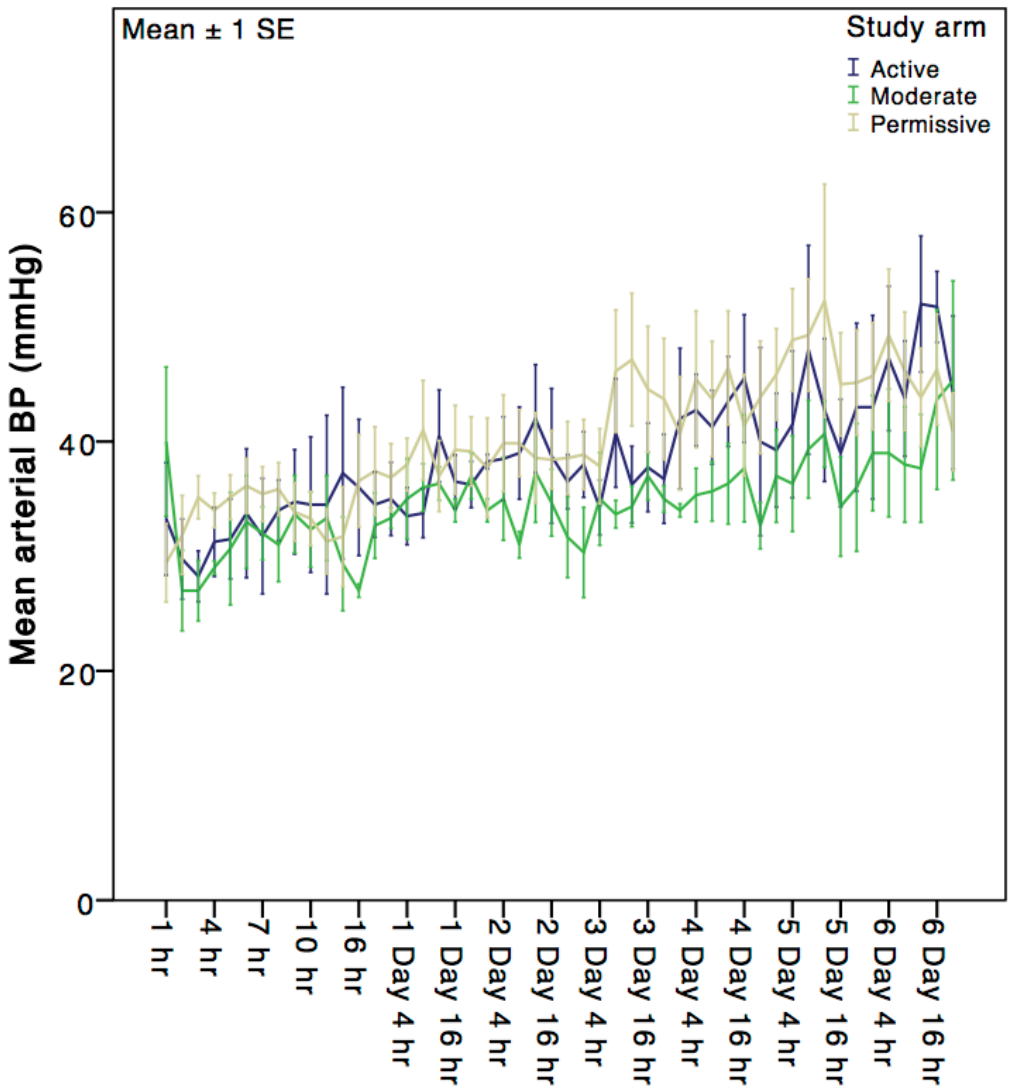


Figure 3.40: Staff recorded mean arterial BP (Invasive and non–invasive) in the three arms of the study during the first week of life

3.8 Blood pressure measurements - discussion

Continuously downloaded invasive mean arterial blood pressure

Mean BP was chosen rather than systolic or diastolic BP because mean BP represents the entire cardiac cycle, it is used to measure the cerebral perfusion pressure (Miall-Allen et al. 1987) and is less likely to be affected by the effect of damping of arterial tracing by small bubbles or clots (Weindling 1989). Moreover, systolic BP is influenced by birth weight and has been found to have higher differential between non–invasive BP and invasive BP (Troy et al. 2009).

Invasive mean arterial BP, being the gold standard, was available for the majority of infants (85%) of infants between 12 hours and 48 hours of age. There was a rise in BP noted in the first few days of postnatal life, consistent with report from previous studies (Fanaroff et al. 2006, Batton et al. 2007, Hegyi et al. 1996, Watkins et al. 1989, Versmold et al. 1981). Across the three arms of the study, BP increased from day 1 to about day 5–6 following which a drop was seen on day 7. This rise of BP between day 5–6 followed by a drop on day 7 is well documented (Tan 1988, Cunningham et al. 1999).

In our study, the rate of rise of mean BP in the first 24 hours was 0.160 mmHg/hour in the Active arm, 0.113 mmHg/hour in the Moderate arm and 0.2 mmHg/hour in the Permissive arm. The average rate of rise of mean BP between the three arms for BP in the first 24 hours in this cohort was 0.158 mmHg/hour. This rate was slightly lower than those reported by other studies (Batton et al. 2014, 2007, Cordero et al. 2002). Batton and colleagues (Batton et al. 2014) in an observational study examined 367 infants born between 23^{+0} to 26^{+6} using both invasive and non–invasive BP monitoring. Invasive arterial lines were available in 83% of infants. BP rose at a rate of 0.2 mmHg/hour between 4 to 24 hours. BP initially was noted to decrease in the first few hours before reaching a nadir at 4 to 5 hours. This decrease could be attributed to the change from non–invasive BP (which overestimated BP) to invasive BP monitoring. Further study by Batton et al (Batton et al. 2007) in a retrospective chart review of 142 infants between 23 to 25 weeks gestation reported the rate of rise in mean BP to be to be 0.3 mmHg/hour during the first 24 hours followed by 0.1 mmHg/hour in the next 24 hours. This study also included a mixture of invasive and non–invasive BP measurements which could explain the higher rate of rise in BP. Cordero and colleagues (Cordero et al. 2002) in a retrospective cohort study examined 101 extremely low birth weight infants with a median gestation of 25 weeks who had

non–invasive and invasive BP monitoring. They demonstrated the rate of rise of mean BP to be 0.25 mmHg/hour during the first 24 hours. The average rate of rise of BP in our cohort was slightly lower than other studies. This could be explained by the quality of BP data which was all invasive and acquired every 10 seconds. Non–invasive BP measurements are known to overestimate BP when compared to invasive BP measurements (Troy et al. 2009, Dannevig et al. 2005, Diprose et al. 1986).

At the start of recruitment, BP in all three arms were similar. The achieved BP on day 1 was highest in the Active arm and lowest in the Permissive arm, thus following the order of randomisation. Subsequently BP rose in all three arms and there were no significant difference between the three arms for the remaining days of the week. Though BP rose across all three arms of the study, it was once again highest in the Active arm and lowest in the Permissive arm on day 6 but this did not achieve statistical significance. For the first 48 hours of life, mean BP in the Active arm had less variability when compared to the Moderate and Permissive arm.

The dispersion of BP data was also examined using SD. We found a significant difference between the three arms in the first 72 hours, when fluctuation in BP is known to cause periventricular haemorrhage, and in the first week of life. Among the three arms, the Active arm had the least SD and the Permissive arm had the highest SD. Cunningham and colleagues (Cunningham et al. 1999) carried out a retrospective study examining invasive BP data from 232 infants with a birth weight of < 1500 grams between born between 1989 to 1995. They found BP to increase in the first week of life. They demonstrated that low BP was significantly associated with intraventricular haemorrhage ($p < 0.05$) and that an increased SD on day 1 is associated with intraventricular haemorrhage on day 2 and 3

of postnatal age. This could perhaps explain why the Active arm had the least amount of intracranial abnormalities in this cohort. Faust et al (Faust et al. 2015) carried out more recent work examining the short term outcomes of very low birth weight infants with hypotension in the first 24 hours using retrospectively collected data from 4907 infants with a gestational age < 32 weeks. They found hypotensive infants to have a higher rate of intraventricular haemorrhage (OR 1.62), bronchopulmonary dysplasia (OR 1.34) and increased mortality (OR 1.90).

Continuously downloaded invasive and non–invasive mean blood pressure

Non–invasive mean BP was performed in a small proportion of infants (<15%) as the majority of infants had invasive mean arterial BP measured. Infants who had non–invasive BP monitoring were of higher gestation when compared to those infants who had invasive BP monitoring. At 4–hourly BP measurements, there was a significant difference between the three arms of the study, following the order of randomisation, between 12–15 hours. This difference was not found in any other time epochs. In contrast to invasive only BP measurements, the Moderate arm had the lowest BP measurements between 48–72 hours and highest BP measurements between 100–144 hours of postnatal life. The Permissive arm had the highest BP measurement at 3 days of postnatal age. This reflects the contribution of the non–invasive BP measurements which is known to overestimate BP (Troy et al. 2009, Dannevig et al. 2005, Diprose et al. 1986).

Examining the validity of oscillometric mean BP measurements measured in our neonatal unit prior to the start of the study showed that non–invasive BP measurements overestimated by 2 mmHg at lower BP values (20 mmHg) when compared with simulator BP measurements. Contrastingly, at higher simulator BP values (60 mmHg) oscillometric BP

measurements were found to under estimate by 1 mmHg. When we compared invasive and non–invasive mean BP in a different cohort of similar infants, we found that non–invasive mean BP measurements taken from the arm overestimated by about 11 mmHg and those taken from the leg overestimated by about 6 mmHg when compared to invasive mean BP. There was proportional bias with non–invasive mean BP measurements taken from the leg overestimating at the higher BP readings. In this cohort (different to the randomised cohort) we did not encounter infants with low mean BP of 20 mmHg as our neonatal unit actively maintained mean BP more than 30 mmHg. Troy and colleagues (Troy et al. 2009) examined 38 extremely low birth weight infants with a median gestation and birthweight of 25 weeks and 754 grams respectively. Of the 148 pairs of BP measurements, oscillometric median mean BP measurements were 12 mmHg higher than mean BP measurements obtained from central arterial lines which was comparable to our results. König et al (König et al. 2012) compared invasive and non–BP measurements using the right arm and leg in sixty infants with a median gestation and birth weight of 26.4 weeks and 924 grams respectively. They divided the infants into three groups based on their birth weight; less than 750 grams, 751–1000 grams and more than 1000 grams. They found that the difference between invasive and non–invasive BP measurements were acceptable but the range of under and over estimation of non–invasive BP measurements were large and inconsistent. They suggested that in the absence of invasive BP measurements to consider using the lower limb for non–invasive measurements in infants less than 1000 grams.

Factors that played a role in the deviance of oscillometric BP measurements when compared to invasive BP included birth weight, current weight and arm circumference. When comparing arm circumference and gestational age, arm circumference was found to be significant factor influencing deviance such that smaller arm circumference was associated

with higher difference between the two methods of measurement. Other variables such as gender, activity level during measurements, ventilatory support and use of umbilical or radial arterial lines were not useful predictors (Dannevig et al. 2005). This results in under treatment of hypotensive preterm infants when using oscillometric BP measurements.

Staff recorded blood pressure

Staff recorded BP involving a mixture of invasive and non–invasive BP measurements showed no significant difference between the three arms of the study at different time intervals. This is likely due to non–invasive BP measurements over-estimating BP. In contrast, staff recorded invasive only BP measurements showed a significant difference between the three arms of the study. Mean BP was highest in the Active arm and lowest in the Permissive arm in the first 24 hours of life for 4–hourly and 12–hourly BP measurements but not for 24–hourly BP measurements. This can be likely explained by effect of averaging. Non–invasive BP measurements during the first 24 hours were all higher than the invasive BP measurements across the three arms of the study for all the different hourly BP measurements. Due to the influence of the non–invasive BP measurements, BP trend during the first week of life showed that BP was highest in the Permissive arm and lowest in the Moderate arm. Permissive BP continued to remain higher than the other two arms for the majority of time. This is in contrast to the BP trend when invasive only BP measurements were considered, where in for the first 24 hours, mean BP was highest in the Active arm and lowest in the Permissive arm. The false elevation of BP due to non–invasive BP measurements should be borne in mind when researchers use staff recorded data for BP related studies and when clinicians use this data to manage the cardiovascular status in the neonatal intensive care unit.

Staff recorded BP measurements are prone to bias with staff potentially likely to record 'higher' BP for the more stable infants and 'lower' BP for the more sicker infants or document the highest or lowest BP in an infant who has borderline BP. Hug and colleagues (Hug et al. 2011) compared clinician recorded invasive mean and systolic BP measurements obtained from Philips healthcare monitors against an automated archiving method from the same invasive monitor from 2,320 adult intensive care records. The primary outcome of this work was to examine the 'consensus' in hypotension, i.e hypotension jointly documented by the nursing staff and the automated archive. They found that at matched levels of specificity (96%), BP from automated method was more sensitive than clinician documented values (28% versus 21%). Likewise for matched level of sensitivity (21%), automated values were more specific than clinician documented values (99% versus 96%). This study concluded that clinician documented BP measurements were inferior when compared to an automated agent with signal quality filtering as early indicators of haemodynamic instability.

3.9 Outcomes of blood pressure intervention levels

Patient characteristics

A total of 134 infants were assessed for eligibility (Figure 3.41) of which 60 (45%) were recruited to the study. As randomisation was achieved using permuted blocks within gestational strata, the number of infants recruited into each arm was not equal. Of the infants recruited to the study, all were followed up and analysed with no infants withdrawing from the study. The baseline patient characteristics (Table 3.14) showed no significant difference in gestational age, birth weight, maternal antenatal steroid administration, mode of delivery, cord gases and temperature on admission between the three study arms.

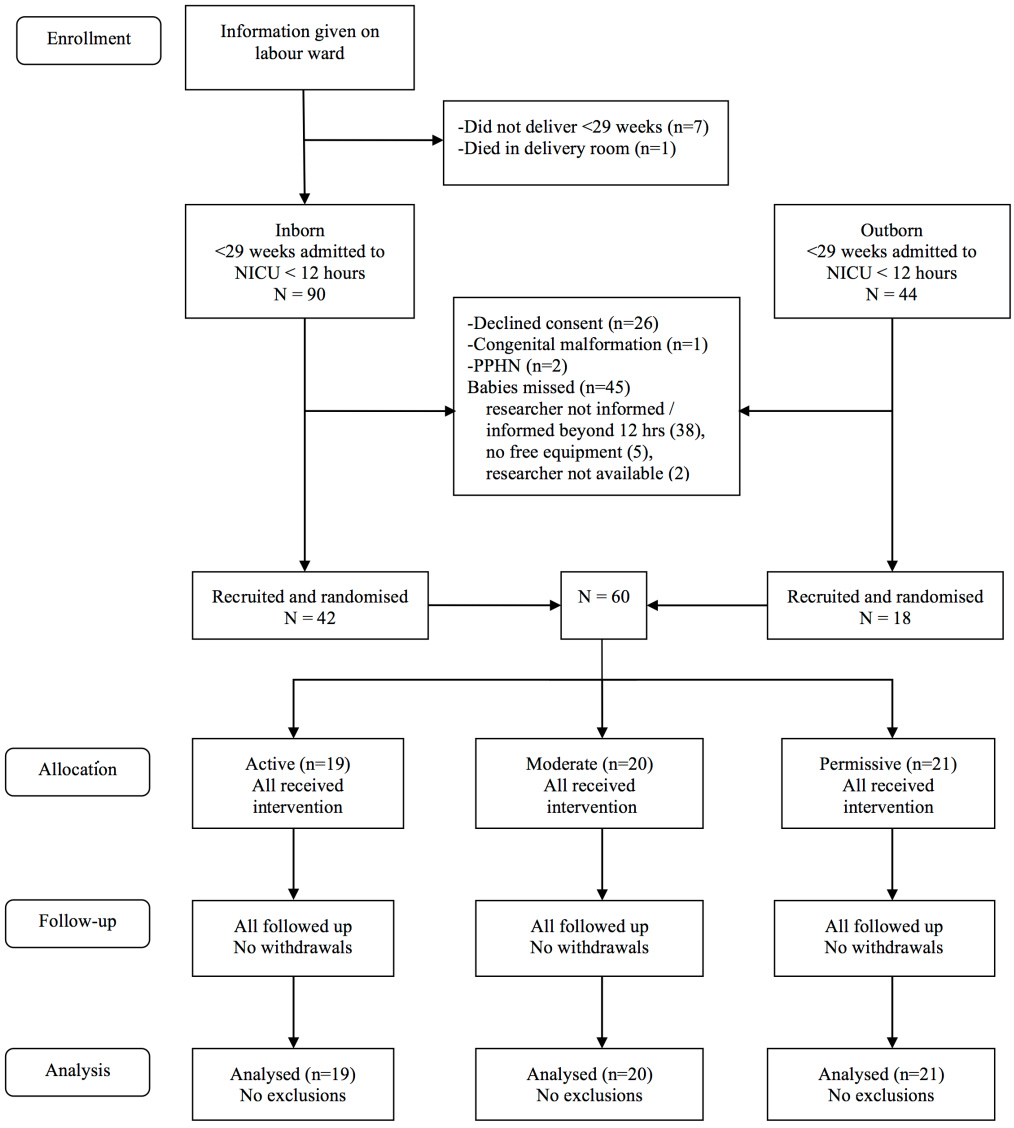


Figure 3.41: Participant flow through the study.

Out of the total of 134 infants assessed for eligibility into the study, 52 infants were excluded. Out of those excluded, twenty-six (50%) parents declined consent for the study. A breakdown of the reasons for declining consent is shown below (Figure3.42).

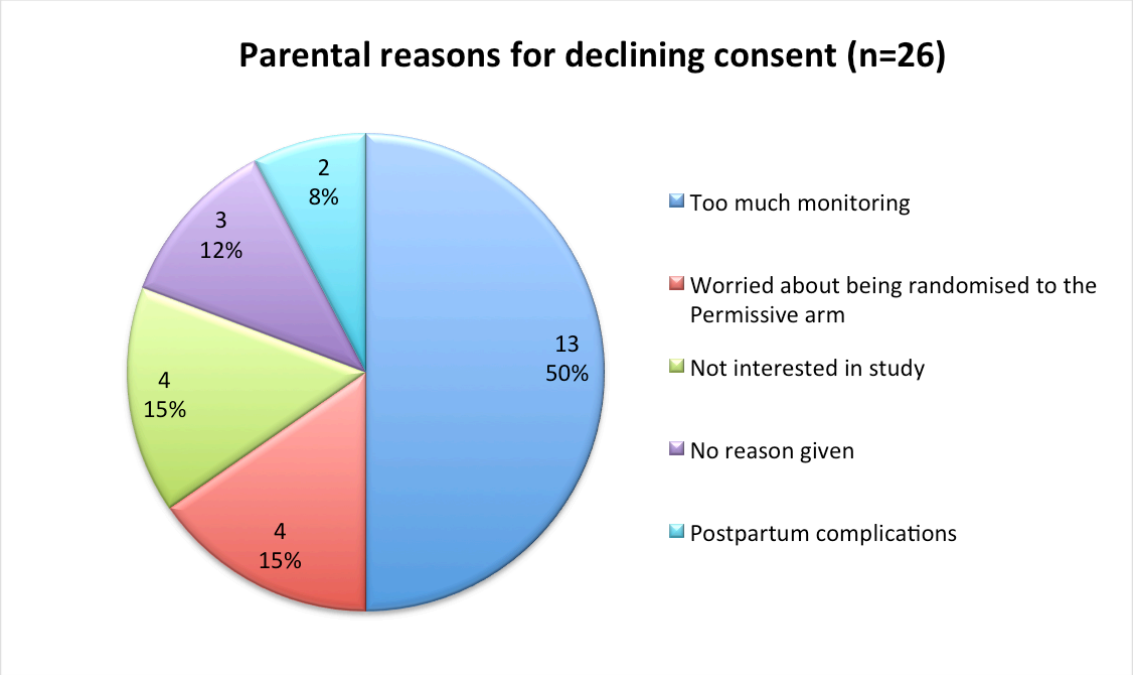


Figure 3.42: Pie chart illustrating the various parental reasons for declining consent for participating in the study.

The most frequent reason given by parents for declining consent to the study was too much monitoring. Parents reported that infants requiring further monitoring/ investigations as part of the study in addition to the ones they would already receive as part of standard care would be overwhelming for a premature infant in the first few days. The second most frequent causes were concerns about being BP not be actively supported if baby were to be randomised to the Permissive arm along with parents not being interested in the study.

Among the 52 infants excluded, 7 (14%) infants who were eligible to participate when initially approached did not deliver before 29 weeks gestation thus becoming ineligible to participate in the study.

	Active N=19	Moderate N=20	Permissive N=21	p value
Gestational age in weeks	25.7 (23.4–28.9)	25.8 (23.3–28.7)	25.6 (23.7–28.7)	0.910
Birth weight in grams, Median (IQR)	760 (705–870)	810 (655–952)	790 (687–970)	0.423
Females, n (%)	11 (58%)	8 (40%)	11 (52%)	0.754
Maternal ethnicity, n (%)				
Black, n=20 (33%)	6 (30%)	5 (25%)	9 (45%)	0.434
Asian, n=17 (28%)	7 (41%)	7 (41%)	3 (18%)	
White, n=15 (25%)	4 (27%)	4 (27%)	7 (46%)	
Other, n=8 (13%)	2 (25%)	4 (50%)	2 (25%)	
Antenatal steroids, n (%)				
No steroids	1 (5%)	2 (10%)	1 (5%)	0.836
Two doses of steroids	16 (84%)	14 (70%)	15 (71%)	
Mothers who received antihypertensive medication, n (%)	4 (21%)	4 (20%)	2 (10%)	0.575
Mode of delivery, n (%)				
Vaginal delivery	15 (79%)	17 (85%)	20 (95%)	0.285
Caesarean section	4 (21%)	3 (15%)	1 (5%)	
Cord gases	n= 6	n= 6	n= 5	
pH	7.32 (7.21–7.40)	7.26 (7.11–7.38)	7.20 (7.09–7.32)	0.115
BE	-4.6 (-12.0, -0.6)	-5.1 (-7.5, -1.5)	-9 (-13.3, -2.8)	0.259
Apgar score				
1 min	5 (0–9)	5 (1–9)	3 (1–9)	0.210
5 min	8 (1–10)	7 (4–10)	7 (3–10)	0.957
Temperature on admission to NICU	36.9 (33.4 –37.8)	36.9 (35.5–37.5)	36.8 (35.8–38.1)	0.658

Table 3.14: Perinatal characteristics at the time of recruitment and randomisation. Where not specified all figures are median (range). There were no significant differences between the group.

Blood Pressure and Inotropic Support

Our initial analysis of intermittent, staff-recorded BP including both invasive and non-invasive measurements did not show any differences in MABP between the groups (Table 3.13) and the trend in BP between the three arms of the study was different compared to invasive only BP data. We found that in the two hours before starting inotropes, staff

recorded a MABP of <19mmHg for 4 infants in the Permissive arm, no infants in the Moderate arm, and 1 infant in the Active arm.

Non–invasive BP measurements tend to overestimate when compared to invasive BP. This tendency was seen in our cohort when infants were changed to non–invasive BP from invasive BP, the subsequent non–invasive BP measurements were on average 11 mmHg higher than the invasive ones. This degree of error was also found when we did further studies comparing invasive and non-invasive BP in another cohort of infants using the same equipment (Figure 3.66). Furthermore when invasive staff-recorded values were compared with continuously downloaded data there were noticeable differences; the 95% limits of agreement were ± 6mmHg, although there was no systematic bias (Figure 3.65). This called into question the use of intermittent BP recordings for further analysis, especially when non-invasive measurements were included.

Therefore, detailed analysis of invasive BP restricted to 51 (85%) infants showed greatest difference in MABP in the first 24 hours as described earlier (Table 3.16). BP trend for the first week of life showed that apart from day 1 there was a separation between the three arms on day 6 but did not achieve statistical significance (Figure 3.43)

Inotropic support was given most frequently to infants recruited to the Active arm (79% received inotropes), and least to infants in the Permissive arm (only 48%, $p<0.05$). However, on examining gestation wise inotrope usage, the majority of 'very immature infants' between 23–24 weeks gestation received inotropic therapy across the three arms whereas 'less immature infants' between 27–28 weeks gestation received less inotropic therapy across the three arms of the study irrespective of the arm to which they were randomised.

For those receiving inotropes, the duration of inotropic therapy was significantly higher in the Active arm and lowest in the Permissive arm but infants receiving inotropes for more than 72 hours was not significantly different between arms. Dopamine was the most frequently used inotrope and this was highest in the active arm and least in the permissive arm ($p<0.05$). The maximum dose of dopamine was not significantly different between the three arms of the study. Dobutamine use not significantly different between the groups. The use of dobutamine was highest in the Permissive arm (14%) followed by the Active arm (10%) and least in the Moderate arm (5%). Among the various cardiovascular support and monitoring parameters compared, the duration of inotropic support was the only parameter that remained significant after carrying out Benjamini–Hochberg correction for multiple testing.

There were no differences in hydrocortisone use for treatment of hypotension or the need for medical or surgical treatment of PDA. The duration of umbilical catheters remaining in–situ was lowest for the Permissive arm and highest in the Moderate arm but this did not achieve statistical significance (Table 3.15).

Patient outcomes	**Active N=19**	**Moderate N=20**	**Permissive N=21**	**p value**
Inotropic support	15 (79%)	11 (55%)	10 (48%)	**0.047**
Inotrope usage by gestation				
23–24 weeks	5/5 (100%)	5/6 (83%)	5/5 (100%)	-
25–26 weeks	8/10 (80%)	4/10 (40%)	4/11 (36%)	
27–28 weeks	2/4 (50%)	2/4 (50%)	1/5 (20%)	
Inotropic agents n (%)				
Dopamine	15 (79%)	11 (55%)	8 (43%)	**0.023**
Dobutamine	2 (10%)	1 (5%)	3 (14%)	0.768
Maximum dose of dopamine (mcg/kg/min)	17 (10 –20)	10 (10–15)	11 (10 –14)	0.097
Duration of inotropes - hours	44 (20–153)	7 (0–73)	0 (0–38)	**0.011***
Inotropes used for >72 hours	8 (42%)	5 (25%)	1 (5%)	0.081
Hydrocortisone for hypotension, n (%)	4 (21%)	3 (15%)	4 (19%)	0.918
Patent ductus arteriosus, n (%)				
Medical treatment (COX inhibitor)	7 (37%)	9 (45%)	8 (38%)	0.949
Surgical ligation	2 (10%)	2 (10%)	5 (24%)	0.477
Duration of UAC - hours	105 (68–232)	108 (55–227)	79 (45–187)	0.223

Table 3.15: Cardiovascular support and monitoring. Values are number (%) or median (IQR), with statistical tests performed for ordered levels (Chi-squared test with liner-by-linear association, Fisher's exact test and Jonckheere-Terpstra test). Duration of inotropic therapy remained significant after Benjamini-Hochberg correction for multiple testing

	Active N=19	**Moderate N=20**	**Permissive N=21**	**p value**
Number of patients with data analysed	17 (89%)	18 (90%)	16 (76%)	
Average MABP 8-11 hours (N 16,16,13)	31.7 (30.8-34.2)	32.3 (29.9-35)	30 (28.4-33.3)	0.128
Average MABP 12-15 hours (N 17,17,16)	33.4 (31.1-36.4)	30.3 (28-33.6)	27.4 (24.9-30.2)	**<0.001**
Average MABP 16-19 hours (N 17,18,16)	34.1 (31.2-37.6)	32 (28.1-33.9)	30.4 (26.8-33)	**0.007**
Average MABP 20-23 hours (N 17,18,16)	35.1 (31.9-37.1)	32.1 (30.2-35.5)	31.7 (29.9-35.5)	0.120
Average MABP 25-48 hours (n 17,18,16)	34 (32.9-36.1)	35.5 (32.9-39.2)	33.4 (31.1-36.1)	0.545
Average MABP 49-72 hours (n 16,13,14)	34.1 (33.5-36.5)	33.6 (31.6-37.5)	32 (27.4-37.3)	0.086
Average MABP 73-96 hours (n 13,13,9)	34.2 (31-35.6)	33.1 (30.8-36.2)	31.9 (26.6-36.8)	0.370
Average MABP 97-120 hours (n 11,10,8)	49 (45.5-53)	46.1 (44.3-52.6)	47 (36.7-54.2)	0.400
Average MABP 121-144 hours (n 10,8,7)	50.1 (46.8-53)	47 (43.2-51.2)	45.6 (37.5-54.7)	0.342
Average MABP 145-168 hours (n 9,8,6)	34.1 (31-39.1)	33.6 (27.7-36.4)	31.5 (27.6-33.6)	0.089

Table 3.16: Mean arterial invasive BP (MABP) measurements 4 hourly from 8 to 24 hours and then 24 hourly for first week of life. Number of infants analysed for each time point shown in parenthesis. Values are median (IQR). Statistical tests performed with Jonckheere-Terpstra test.

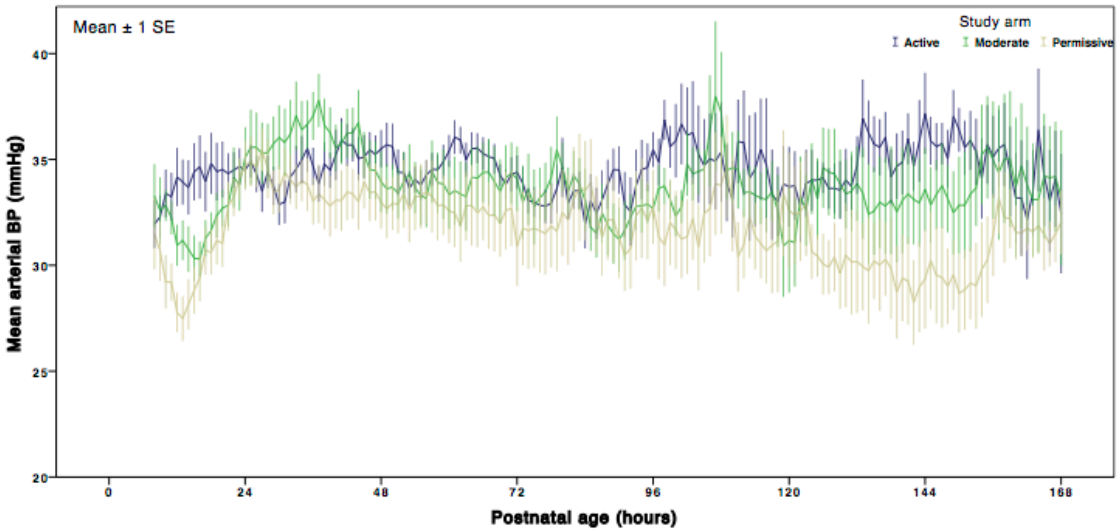


Figure 3.43: Invasive mean arterial BP in the three arms of the study from 8 hours to 168 hours.

Blood pressure and markers of peripheral perfusion

The relationship between cardiac output and the markers of peripheral perfusion has been explored in section 3.3.1.

The most frequently markers of peripheral perfusion in clinical practice such as capillary refill time, base excess, serum lactate, urine output and cardiac output were not significantly different between the three arms of the study both on day 1 and day 3 (Table 3.18). There were no significant correlations between invasive BP and the majority of markers of peripheral perfusion on day 1 and day 3 (Table 3.17). Invasive mean arterial BP was directly related to urine output (Figure 3.44) on day 1 and inversely related to serum lactate on day 3 (Figure 3.45).

Markers of circulatory failure	Invasive mean arterial BP (mmHg)	
	Day 1	**Day 3**
Capillary refill time (seconds)	0.084 (0.560)	0.286 (0.074)
Serum lactate (mmol/L)	-0.129 (0.371)	-0.415 **(0.008)**
Urine output (ml/kg/hr)	0.393 **(0.005)**	-0.084 (0.613)
Cardiac output (ml/kg/min)	-0.006 (0.966)	-0.069 (0.673)

Table 3.17: Invasive BP correlated against the various markers of circulatory failure on Day 1 and 3. Pearson's correlation used. Figures represent correlation, r with p–value in parenthesis.

Markers of peripheral perfusion on Day 1 and 3	**Active N=19**	**Moderate N=20**	**Permissive N=21**	**p value**
Capillary refill time (seconds)				
Day 1	2 (2–3)	2 (2–3)	2 (2–3)	0.904
Day 3	2 (2–3)	2 (2–2)	2 (2–2)	0.700
Base excess (mEq/L)				
Day 1	-4.2 (-6.4, -2.4)	-4.7 (-7.8, -2.3)	-3.4 (-4.8, -1.9)	0.493
Day 3	-4.5 (-5.4, -2.9)	-6.2 (-6.9, -4.5)	-5.6 (-7.0, -4.9)	0.053
Serum lactate (mmol/L)				
Day 1	2.4 (1.7–3.5)	2.2 (1.8–3.8)	2.4 (1.7–3.2)	0.940
Day 3	1.6 (1.4–2.7)	1.9 (1.5–2.1)	1.8 (1.3–2.1)	0.512
Urine output (ml/kg/min)				
Day 1	3.0 (1.7–4.6)	3.5 (2.6–4.5)	2.6 (1.7–3.6)	0.181
Day 3	4.4 (3.0–5.6)	4.2 (3.0–5.0)	4.6 (3.6–5.8)	0.763
Cardiac output (left ventricular output, ml/kg/min)				
Day 1	166 (159–220)	160 (125–195)	181 (144–202)	0.582
Day 3	210 (147–256)	220 (164–250)	200 (172–251)	0.667

Table 3.18: Markers of peripheral perfusion including cardiac output measured on Day 1 and Day 3 of life. Continuous variables are expressed as median (IQR). Statistical test performed with Jonckheere-Terpstra test and ANOVA with contrasts.

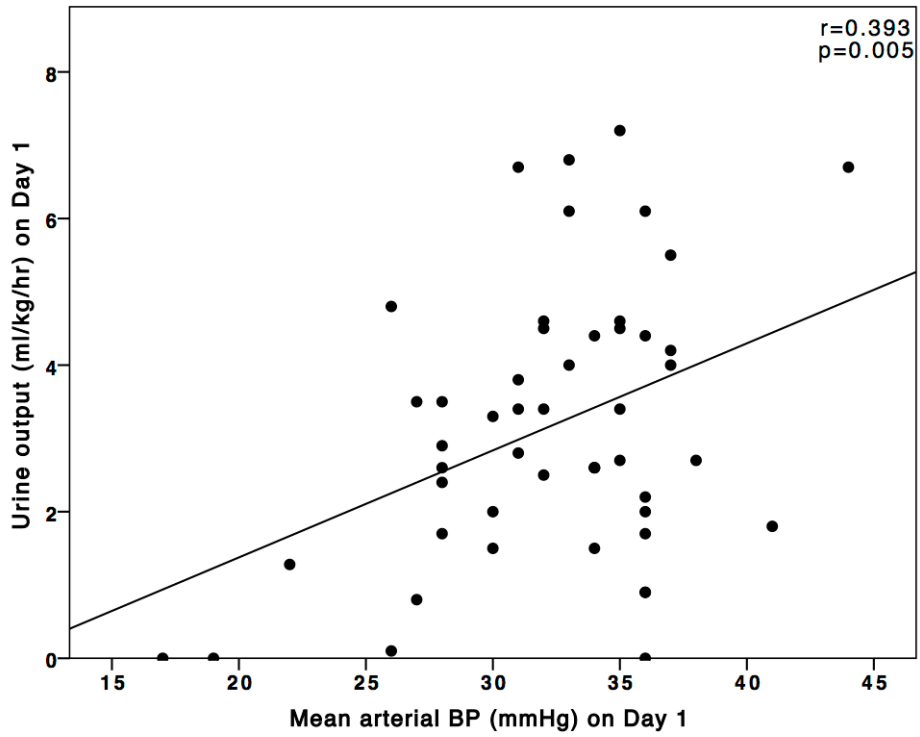


Figure 3.44: Relationship between invasive mean arterial BP and urine output on Day 1. Pearson's correlation used.

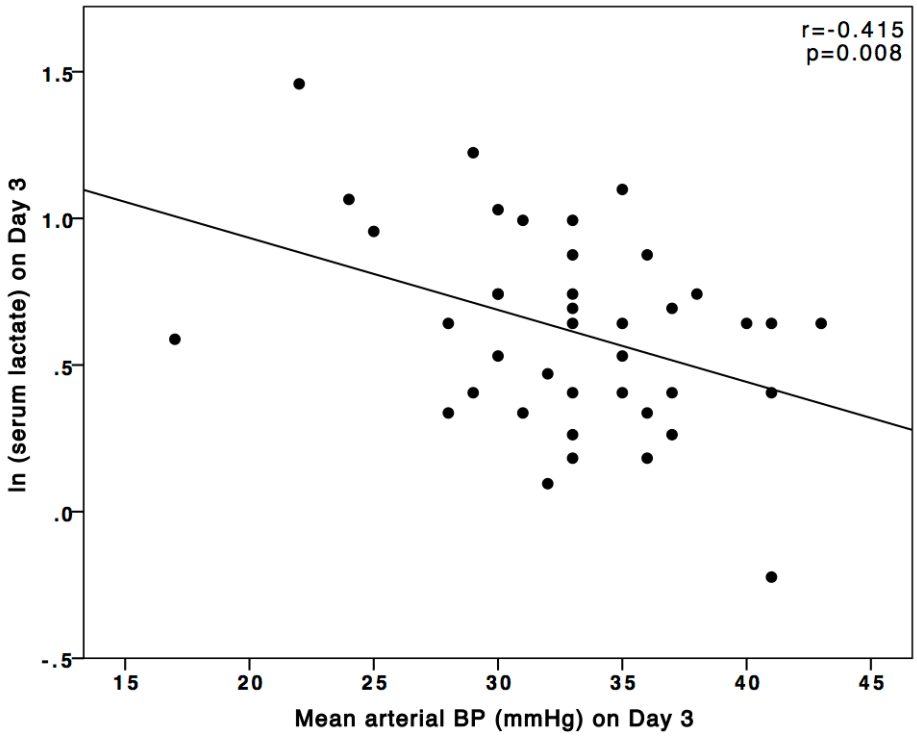


Figure 3.45: Relationship between invasive mean arterial BP and natural log scale of serum lactate on Day 3. Pearson's correlation used.

In addition to the above mentioned parameters, renal function in terms of maximum serum potassium and serum creatinine measured in the first 2 weeks of life was examined against the invasive BP measured on day 1 and 3. Serum potassium was significantly negatively associated with BP on day 3 only (Figure 3.46). However, serum creatinine was negatively (non–significant) associated with BP on both days (Figure 3.47). Gestational age was inversely related to serum potassium levels consistently on day 1 (Table 3.19).

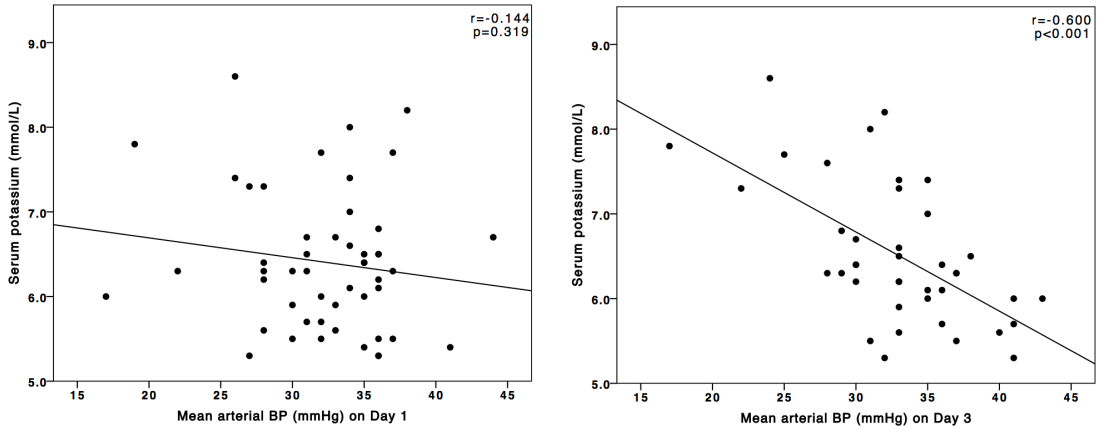


Figure 3.46: Relation between BP and potassium on day 1 and 3 of postnatal life. Pearson's correlation used.

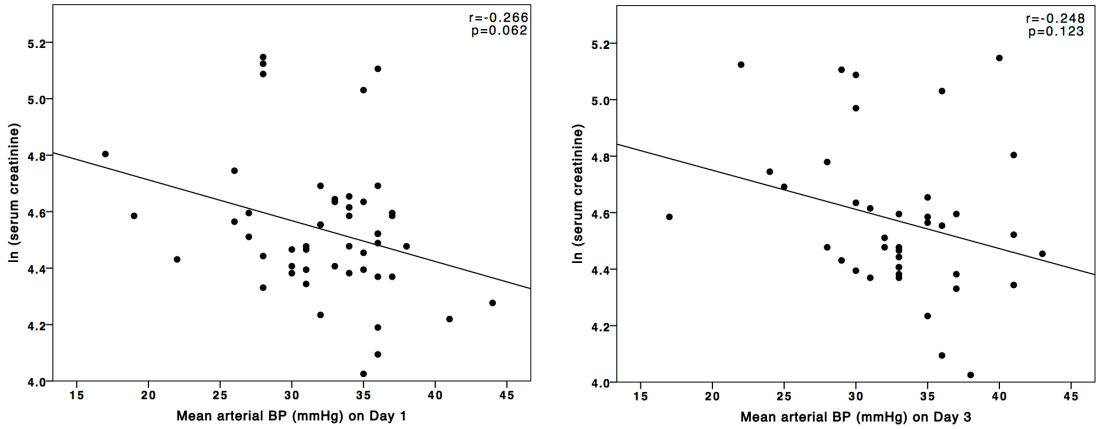


Figure 3.47: Relation between BP and creatinine on day 1 and 3 of postnatal life. Pearson's correlation used.

Clinical parameters	Day 1 (N =48)		Day 3 (N = 38)	
	Serum potassium (mmol/L)§	Serum creatinine (mmol/L)§	Serum potassium (mmol/L)§	Serum creatinine (mmol/L)§
Gestation (weeks)	-0.498***	-0.268	-0.386*	-0.137
Invasive MABP (mmHg)	-0.144	-0.266	-0.600***	-0.248
Cardiac output (ml/kg/min)	0.029	0.079	0.223	0.013
PDA dimeter [colour] (mm) (day 3)§	-0.252	-0.076	0.242	0.127
Max dose of dopamine (mcg/kg/min)§	0.286*	0.261	0.169	0.177

Table 3.19: Relationship between renal parameters and various clinical characteristics. Correlation between variables examined using Pearson's correlation and Spearman's rho (§) (* p <0.05, ** p < 0.01, *** p < 0.001).

Stepwise backward linear regression showed that serum potassium was significantly related to gestation on day 1 and to BP only on day 3 (Table 3.20) after adjusting for gestation, invasive mean arterial BP, cardiac output, PDA diameter (colour) and maximum dose of dopamine. Serum creatinine was only related to gestation after adjusting for the above mentioned variables on day 3 (Table 3.21).

Clinical parameters	Day 1 (N =48)				Day 3 (N = 38)			
	Unstandardized B coefficients	(95%CI)	p value	Tolerance (VIF)	Unstandardized B coefficients	(95%CI)	p value	Tolerance (VIF)
Full model								
Gestation	-0.22	(-0.39, -0.05)	**0.013**	0.79 (1.3)	-0.082	(-0.283, 0.119)	0.412	0.75 (1.3)
Invasive MABP	0.017	(-0.028, 0.063)	0.447	0.84 (1.2)	-0.087	(-0.134, -0.039)	**0.001**	0.77 (1.3)
Cardiac output	0.001	(-0.003, 0.005)	0.662	0.92 (1.1)	0.001	(-0.003, 0.005)	0.58	0.80 (1.2)
PDA dimeter [colour]	-0.367	(-0.72, -0.02)	**0.04**	0.93 (1.1)	0.145	(-0.222, 0.513)	0.427	0.83 (1.2)
Max dose of dopamine	0.025	(-0.01, 0.05)	0.108	0.83 (1.2)	-0.004	(-0.038, 0.031)	0.824	0.84 (1.2)
Stepwise condensed model after elimination of non–significant variables								
Gestation	-0.255	(-0.408, -0.101)	**0.002**	0.98 (1.0)	-	-	-	-
Invasive MABP	-	-	-	-	-0.093	(-0.135, -0.052)	**<0.001**	1 (1)

Table 3.20: Stepwise backward multiple regression analysis between serum potassium and clinical parameters on day 1 and 3.

Clinical parameters	Day 1 (N =48)				Day 3 (N = 38)			
	Unstandardized B coefficients	(95%CI)	p value	Tolerance (VIF)	Unstandardized B coefficients	(95%CI)	p value	Tolerance (VIF)
Full model								
Gestation	-3.924	(-10.18, 2.33)	0.212	0.79 (1.3)	-2.692	(-11.65, 6.27)	0.545	0.75 (1.3)
Invasive MABP	-0.802	(-2.46, 0.85)	0.333	0.84 (1.2)	-0.95	(-3.08, 1.18)	0.369	0.77 (1.3)
Cardiac output	0.021	(-0.14, 0.18)	0.792	0.92 (1.1)	-0.043	(-0.21, 0.12)	0.606	0.80 (1.2)
PDA dimeter [colour]	-0.319	(-13.09, 12.45)	0.96	0.93 (1.1)	6.329	(-10.05, 22.71)	0.437	0.83 (1.2)
Max dose of dopamine	0.598	(-0.51, 1.71)	0.283	0.83 (1.2)	0.321	(-1.22, 1.86)	0.674	0.84 (1.2)
Stepwise condensed model after elimination of non–significant variables								
Gestation	-6.039	(-11.50, -0.58)	**0.031**	1 (1)	-	-	-	-

Table 3.21: Stepwise backward multiple regression analysis between serum creatinine and clinical parameters on day 1 and 3.

Markers of peripheral perfusion	Invasive mean arterial BP (mmHg)	
	Correlation on Day 1	Correlation on Day 3
Capillary refill time (seconds)	+	+
Serum lactate (mmol/L)	-	- **
Urine output (ml/kg/hr)	+ **	-
Serum potassium (mmol/L)	-	- ***
Serum creatinine (mmol/L)	-	-

Table 3.22: Summary table showing the direction of correlation between invasive BP against the various markers of peripheral perfusion on day 1 and 3. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

Markers of peripheral perfusion	Cardiac output (ml/kg/min)	
	Correlation on Day 1	Correlation on Day 3
Capillary refill time (seconds)	-	-
Serum lactate (mmol/L)	-	+
Urine output (ml/kg/hr)	+	+
Serum potassium (mmol/L)	-	+
Serum creatinine (mmol/L)	+	-

Table 3.23: Summary table showing the direction of correlation between cardiac output against the various markers of peripheral perfusion on day 1 and 3. No statistically significant relationships.

Haemodynamic and electroencephalographic parameters

All infants studied had detailed echocardiography and aEEG performed simultaneously on Day 1 at a median (IQR) age of 18 (12–22) hours and on Day 3 at a median (IQR) age of 77 (69–91) hours. Normal cardiac structure was confirmed with initial echocardiography. Physiological measurements like left ventricular output, shunt across the patent ductus arteriosus, right common carotid artery blood flow, superior mesenteric artery blood flow and aEEG parameters were measured using methods described earlier on all infants.

There were no differences in left ventricular output, presence of patent ductus arteriosus, common carotid artery blood flow volumes between the three arms of the study on Day 1 and 3. Superior mesenteric artery blood flow was significantly different on Day 3 with no trend. After correction for multiple testing with Benjamini-Hochberg correction, there were no statistically significant effects of any parameter in the three arms of the study. All electroencephalographic parameters, both on Day 1 and 3, did not differ significantly between the three arms (Table 3.24).

Physiological characteristics on Day 1 and 3	**Active N=19**	**Moderate N=20**	**Permissive N=21**	**p value**
Circulatory parameters				
Cardiac output (left ventricular output, ml/kg/min)				
Day 1	166 (159–220)	160 (125–195)	181 (144–202)	0.582
Day 3	210 (147–256)	220 (164–250)	200 (172–251)	0.667
Patent ductus arteriosus present (n (%))				
Day 1	18 (95%)	19 (95%)	17 (81%)	0.346
Day 3	12 (63%)	13 (68%)	15 (71%)	0.581
Common carotid artery blood flow (ml/kg/min)				
Day 1	12 (10–14)	12 (8–14)	12 (9–16)	0.406
Day 3	15 (12–18)	13 (12–15)	15 (11–20)	0.660
Superior mesenteric artery blood flow velocity (mean of peak velocity envelope - cm/s)				
Day 1	21 (16–29)	29 (15–35)	21 (17–28)	0.840
Day 3	21 (14–27)	30 (24–40)	26 (22–31)	**0.041**
Electroencephalographic parameters				
Maximum aEEG amplitude in μV				
Day 1	12.0 (9.8–14.3)	10.0 (7.3–14.4)	13.1 (9.6–15.5)	0.445
Day 3	15.0 (8.3–20.6)	16.0 (10.0–19.8)	13.0 (12.9–18.8)	0.816
Minimum aEEG amplitude in μV				
Day 1	3.1 (2.8–3.9)	2.5 (2.0–3.7)	3.5 (2.5–3.9)	0.613
Day 3	3.5 (2.4–5.1)	4.0 (2.9–5.2)	4.2 (3.4–5.3)	0.869
Median discontinuity in seconds				
Day 1	20 (15–26)	25 (17–37)	17 (9–30)	0.414
Day 3	9.5 (1.5 –31.9)	11 (6–23.7)	8.5 (0–18.6)	0.495

Table 3.24: Circulatory and electroencephalographic parameters measured on Day 1 and Day 3 of life. Continuous variables are expressed as median (IQR). After Benjamini-Hochberg correction for testing of multiple physiological outcome measures, there were no statistically significant effects of study arm on any parameter.

Clinical outcomes

There were no statistically significant differences in general clinical outcomes including mortality, respiratory complications, gastrointestinal complications, retinopathy or renal parameters and duration of care between the three arms of the study (Table 3.25).

Clinical outcomes	**Active N=19**	**Moderate N=20**	**Permissive N=21**	**p value**
Mortality				
Death before discharge from hospital, n (%)	4 (21%)	3 (15%)	2 (10%)	0.540
Causes of death				
- Perinatal asphyxia	1	-	-	
- Periventricular haemorrhage	-	1	-	
- Late onset infection	1	2	-	
- Necrotising enterocolitis	1	-	1	
- Chronic lung disease	-	-	1	
- Other (subcapsular liver haematoma)	1	-	-	
Respiratory complications				
Bronchopulmonary dysplasia (BPD), n (%)	13 (68%)	13 (65%)	12 (57%)	0.504
Dexamethasone for BPD, n	3 (16%)	1 (5%)	5 (24%)	0.265
Gastrointestinal complications				
Necrotizing enterocolitis (NEC) n (%)				
Medical treatment	3 (16%)	4 (20%)	5 (24%)	0.920
Surgical treatment	2 (10%)	0 (0%)	1 (5%)	0.305
Gastrointestinal complications				
Non - NEC perforation, n (%)	0 (0%)	1 (5%)	2 (9%)	0.767
Retinopathy of prematurity				
Stage 3 ROP, n (%)	3 (16%)	2 (10%)	2 (9%)	0.973
Need for laser treatment	3 (16%)	0 (0%)	2 (9%)	0.339
Renal parameters during first week of life				
Maximum serum creatinine in mmol/L	88 (76–104)	90 (83–102)	98 (80–113)	0.127
Maximum serum potassium in mmol/L	6.3 (5.9–6.7)	6.3 (5.6–7.3)	6.1 (5.4–6.6)	0.741
Duration of care by BAPM level				
Neonatal intensive care days	29 (13–49)	27 (9–43)	32 (15–56)	0.916
High dependency care days	38 (26–50)	36 (1–48)	34 (17–54)	0.779
Special care days	23 (5–40)	18 (2–40)	23 (15–28)	0.692

Table 3.25: Other clinical outcomes; there were no statistically significant differences between groups. Continuous variable are expressed as median (IQR). BPD defined as oxygen dependency or need for respiratory support at 36 weeks post-conceptual age.

None of the infants in the active arm developed non–NEC perforation compared to two infants in the Permissive arm. Serum creatinine was lowest in the active arm and highest in the Permissive arm. Though there was a clear trend seen in these two parameters, none of these achieved statistical significance.

Cranial ultrasound findings

The consensus in decision for worst grade cranial ultrasound abnormalities between the two blinded reviewers was 92% with consensus achieved in 100% of cases after discussion. The predefined outcome of parenchymal periventricular hemorrhage on cranial ultrasound occurred in only 2/60 infants, which meant that statistical comparisons between groups could not be carried out. A normal cranial ultrasound scan was found most often in the Active arm (84%) and least in the Moderate arm (60%). The rates of Grade 2-4 PVH were significantly different between the groups (p=0.008), being highest in the Moderate arm (30%) and lowest in the Active arm (0) (Table 3.26).

The composite outcome of death or parenchymal brain abnormality (pre-defined outcome) was similar between the groups. The composite outcome of Grade 2-4 PVH, PVL or parenchymal cysts was significantly different between the groups with it being highest in the Moderate arm (6, 30%) and lowest in the Active arm (0). There was no dose-response effect, as the worst outcomes were found in the Moderate arm.

The composite outcomes of intraventricular or parenchymal PVH and Grade 2-4 PVH, PVL or parenchymal cysts remained statistically significant even after Benjamini–Hochberg correction for multiple testing.

Invasive BP monitoring was available for all infants with periventricular haemorrhage in the first week of life and for the composite outcomes on day 1 and only two infants had non–invasive BP monitoring on day 3 of postnatal life.

	Active N=19	**Moderate N=20**	**Permissive N=21**	**p value**
Periventricular Haemorrhage (PVH) in first week of life				
Normal–No PVH	16 (84%)	12 (60%)	16 (76%)	0.219
Subependymal Haemorrhage	3 (16%)	2 (10%)	4 (19%)	-
IVH (no dilatation)	0	3 (15%)	1 (5%)	-
IVH with dilatation	0	1 (5%)	0	-
Haemorrhagic parenchymal infarct [#]	0	2 (10%)	0	-
Late findings				
Cystic Periventricular Leukomalacia (PVL) [#]	0	0	0	-
Porencephalic cyst	0	2	1	-
Ventricular dilatation	1	1	1	-
Composite outcomes				
Death or parenchymal brain abnormality [#]	4 (21%)	4 (20%)	3 (14%)	0.841
Intraventricular or parenchymal PVH	0	6 (30%)	1 (5%)	**0.008**
Grade 2–4 PVH, PVL or parenchymal cysts	0 (0%)	6 (30%)	2 (10%)	**0.014**

Table 3.26: Cranial ultrasound findings. ([#] pre-specified outcome measure, PVH periventricular haemorrhage, IVH intraventricular haemorrhage, PVL periventricular leukomalacia). Differences were assessed using Fisher's exact test, and remained significant after Benjamini-Hochberg correction for multiple testing.

Among the three groups, a total of 16 infants who had cranial ultrasound abnormalities in the first week of life, underwent further analysis. The baseline characteristics of infants who had periventricular haemorrhage and those that did not are compared (Table 3.27). Infant with periventricular haemorrhage were of a significantly lower gestation and birthweight when compared to infants who had normal cranial ultrasound scans. Mean BP (from 8 to 72 hours –as this was when fluctuations in BP were more likely to cause in-

tracranial pathology) between the two group of infants was significantly different (Figure 3.48) with infants with periventricular haemorrhage having lower BP and higher percentage of inotropic support compared to infants who had normal cranial ultrasound scans.

Clinical parameters	**Infants with periventricular haemorrhage**	**Infants with normal cranial ultrasound**	**p - value**
N (%)	16 (27%)	44 (73%)	–
Gestation in weeks	24.6 (1.2)	26.2 (1.3)	**< 0.001**
Birth weight in grams	711.2 (199.8)	855.6 (172.3)	**0.017**
Mean (Day 1 and 3) $PaCO_2$ in KPa	5.7 (1.4)	5.3 (1.0)	0.268
Antenatal steroids, N (%)	15 (94%)	41 (93%)	0.713#
Inotropic support, N (%)	11 (69%)	17 (39%)	**0.047***
Mean arterial BP (8–72 hrs) in mmHg	33.2 (4.4)	37.0 (6.0)	**0.013**

Table 3.27: Characteristics of infants with and without periventricular haemorrhage during the first week of life. Where not specified, all figures are mean (SD). All statistical analysis performed using independent samples t test. * Pearson's Chi Square test and # Fisher's exact test used.

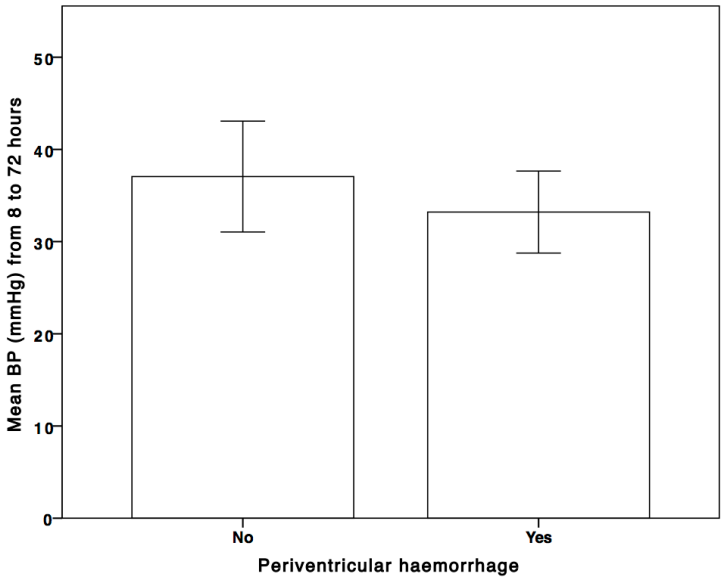


Figure 3.48: Mean BP from 8 to 72 hours in infants with and without periventricular haemorrhage during the first week of life. Error bar represents ±1SD.

Comparison of infants with the composite outcome of significant periventricular haemorrhage, ventricular dilatation, periventricular leucomalacia and periventricular cysts versus

infants with normal cranial ultrasound showed that those with cranial ultrasound abnormalities had significantly lower mean (SD) BP of 32.8 (3.9) mmHg when compared to infants with normal cranial ultrasound 36.7 (6.0) mmHg (p = 0.027, Independent samples t test) (Figure 3.49). Infants with cranial ultrasound abnormalities were of a significantly lower gestation and birth weight as well.

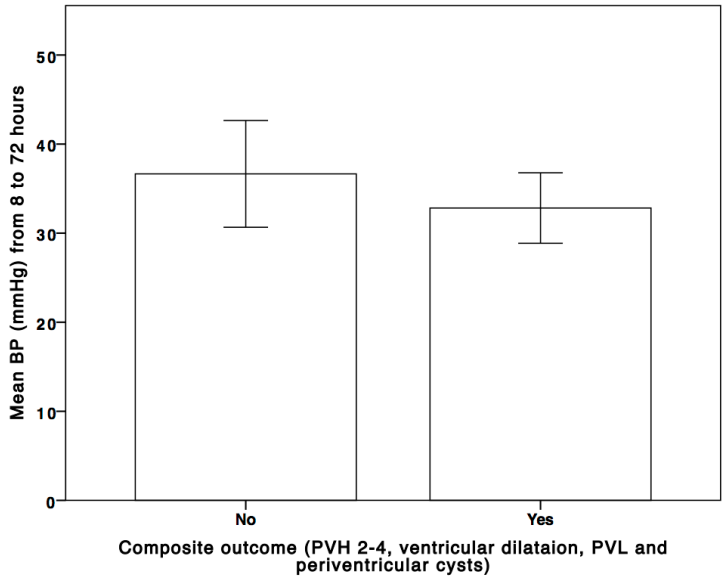


Figure 3.49: Mean BP from 8 to 72 hours in infants with and without the composite outcome of cranial ultrasound abnormalities. Error bar represents ±1SD.

Using binary logistic regression, we found that gestation was the only factor (p = 0.015) which predicted periventricular haemorrhage after adjusting for gestation, birthweight, inotrope administration, $PaCO_2$ and mean BP (8 to 72 hours) in these 16 infants. However, when considering all the infants in this study, those infants whose BP was actively supported (Active arm) had no cranial ultrasound abnormalities when compared with the other arms of the study.

3.10 Outcomes of blood pressure intervention levels - discussion

3.10.1 Clinical outcomes of the blood pressure intervention levels

The gestational age and birth weight were not significantly different between the three arms of the study. A similar number of mothers received two doses of antenatal steroids. Antihypertensive medication given to mother was lower in the Permissive arm but this was not statistically significant. There were no significant differences in the mode of delivery, cord gas, Apgar score or on the temperature on admission to the neonatal uint.

The different aspects such as physiological parameters measured and BP measurements were discussed so far. In the following pages, I will discuss the main clinical outcomes under different sections from this study.

Inotropic support

We found that infants with a lower gestational age and birth weight required more inotropic support when compared to infants of a higher gestational age and birth weight, a finding consistently reported in studies examining this relationship (Batton et al. 2016, 2013, Dempsey et al. 2009, Batton et al. 2007, Laughon et al. 2007, Fanaroff et al. 2006, Cunningham et al. 1999). Inotropic support was similar in the Moderate and Permissive arms but higher in the Active arm. When gestation wise analysis of inotropic support was examined, this showed that the majority (100%) of infants below 26 weeks gestation required inotropic support irrespective of the arm that the infants were randomised to. In contrast, a smaller percentage (50%) of infants more than 27 weeks gestation across the three arms received inotropic support. Laughon and associates (Laughon et al. 2007) reported that inotropic support is more likely to be required for less mature infants than

more mature infants, > 90% for infants at 23 weeks gestation when compared to 73% for infants at 27 weeks gestation. In addition to the gestation, inter institution variability (anti–hypotensive treatment varied between 29% to 98% whereas vasopressor use varied between 6% to 64%) playing a role for this difference in the management of BP (after adjusting for infants characteristics and lowest mean arterial BP on the day of treatment).

Dopamine, one of the most frequently used inotropic agent (Gupta and Donn 2014, BAPM 1992), was the most commonly used inotropic agent. The number of infants receiving dopamine was significantly higher in the Active arm (79%) and lowest in the Permissive arm (43%). The median maximum dose of dopamine was highest in the Active arm (17 mcg/kg/min) and lowest in the Moderate arm (10 mcg/kg/min). The duration of inotropic support was significantly different between the three arms; with a median duration of 44 hours in the Active arm, 7 hours in the Moderate arm and 0 hours in the Permissive arm ($p = 0.011$). This difference between the arms remained significant after correcting for multiple testing. Dobutamine, the second inotropic agent, was most frequently used in the Permissive arm. The inotropic use for > 72 hours was highest in the Active arm and lowest in the Permissive arm. Though there was a clear trend noted, this was not statistically significant.

Markers of circulatory failure

The commonly used markers of circulatory failure such as capillary refill time, serum lactate, urine output and heart rate (de Boode 2010) to identify low flow states were not significantly different between the arms of the study.

There was poor correlation between the various markers of peripheral perfusion and car-

diac output in this cohort as previously reported. Capillary refill time was negatively correlated to cardiac output and positively correlated to BP on both days in this cohort. Capillary refill time (LeFlore and Engle 2005) and base excess (Deshpande and Platt 1997, Kluckow and Evans 2001*a*) have been shown to be an unreliable indicators of circulatory failure. The various markers of circulatory failure when used in combination along with BP has been shown to have a higher sensitivity than when used on its own. Osborn and associates (Osborn 2004) showed that mean BP $\leq$ 30mmHg along with a central capillary refill time of $\geq$ 3 seconds has a 78% sensitivity of identifying infants with low blood flow. These markers correlated with invasive mean arterial BP both on day 1 and day 3.

Serum lactate was negatively correlated to cardiac output on day 1 and found to have no relation on day 3. BP was negatively correlated to serum lactate on both days. Serial serum lactate measurements has been used in prognostication (Deshpande and Platt 1997) in ventilated infants and raised serum lactate on its own is predictive of low flow states and in conjunction with capillary refill time has been found to have increased sensitivity low flow states (Miletin et al. 2009).

Renal parameters in terms of urine output and serum electrolytes were also investigated as markers of circulatory failure. Urine output was directly related to BP on both days and with cardiac output on day 1. Serum potassium and creatinine were negatively related to BP on both days but cardiac output was negatively related with serum potassium on day 1 only. Though BP remained a significant predictor of serum potassium on day 3 only after adjusting for other variables including gestation, we found that gestation remained a strong predictor of serum potassium and creatinine on day 1. Previous studies examining the relation between serum creatinine and gestational age have produced gestation

and birth weight based reference charts (Thayyil et al. 2008). Serum creatinine was negatively correlated with BP but between the three arms there was a clear non–significant trend noted in serum creatinine levels, with it being highest in the Active arm and lowest in the Permissive arm. Low cardiac output and BP is associated with hyperkalaemia, a finding found by other investigators (Kluckow and Evans 2001*a*, 1998). Kluckow and associates (Kluckow and Evans 2001*a*) have shown that low flow states are associated with low urine output and hyperkalaemia, a finding that was seen in this cohort on day 1. Thayyil and colleagues (Thayyil et al. 2008) examining 125 infants below 28 weeks found that hyperkalaemia peaked on day 3 with no difference found in birth weight or gestation between the hyperkalaemic and normokalaemic group. They also found no significant difference between the gestation. Further work by Kluckow et al (Kluckow and Evans 1998) examined the relationship between low systemic flow using SVC and early changes in serum lactate and potassium levels on 126 infants with a mean gestation of 27 weeks. They found a significant relationship between the mean and peak potassium level in the first 24 hours and lowest measured levels of SVC flow. They concluded that when comparing lactate and hyperkalaemia, hyperkalaemia remained a more specific marker of hypoperfusion as shown in this work.

Blood pressure and markers of peripheral perfusion

The majority of infants have invasive arterial lines (51 infants) and therefore arterial blood gas analysed in this study. A small proportion of infants who did not have arterial lines had capillary blood gas analysis performed. There is a good correlation between arterial and capillary blood for all blood gas parameters except for PaO_2 in the presence of hypotension where a poor correlation exists between arterial and capillary blood (Yildizdaş et al. 2004).

BP was not significantly related to capillary refill time on both days. Serum lactate was negatively correlated to BP, to a significant level on day 3 only. BP was significantly positively correlated to urine output on day 1. Serum potassium levels were negatively correlated to BP on both days. Mean BP remained significantly related to serum potassium levels on day 3 after adjusting for gestational age, cardiac output, PDA colour diameter and maximum dose of dopamine. We found serum creatinine to have a negative correlation with BP but this did not persist after adjusting for the above factors. Only gestational age remained a significant predictor of serum creatinine levels on day 1.

Haemodynamic and electroencephalographic parameters

Physiological measurements such as left ventricular output, common carotid artery blood flow, superior mesenteric artery blood flow and incidence of patent ductus arteriosus were not significantly different between the three arms of the study. However, there was a significant increase in all the physiological parameters between day 1 and day 3 as explained earlier.

As for electroencephalographic measurements, such as maximum and minimum EEG amplitude and median discontinuity, there were no significant difference between the three arms. All electroencephalographic measurements increased significantly from day 1 to day 3 as explained earlier.

Clinical outcomes

Being a pilot study, one of the purpose of carrying out such a study was to explore and identify variables which could be examined later using larger trials. Hence several secondary outcomes were included. Though this study was not powered to detect significant differ-

ences in complications, we found no significant difference in respiratory, gastrointestinal, renal parameters, retinopathy of prematurity, level of BAPM level care and mortality between the three arms of the study. There was a clear trend in serum creatinine, with it being lowest in the Active arm and highest in the Permissive arm, this did not achieve statistical significance. Serum potassium did not differ significantly between the arms of the study. Similarly, there was a trend noted in non–NEC gastrointestinal perforation. This was highest in the Permissive arm, followed by the Moderate arm and none in the Active arm. The infants who received medical treatment for PDA was highest in the Permissive arm and lowest in the Active arm but when it came to surgical treatment, infants in the Active arm were highest, followed by the Permissive arm and the Moderate arm.

There were no significant difference between the three arms of the study in terms of mortality either. Though there was a higher rate of death in the Active arm, the causes of these deaths illustrate that these were not related to the arm that the infants were randomised to. This is contrary to the finding from several studies which have implicated proactively treating low BP to be associated with poorer outcome (Batton et al. 2016, Fanaroff et al. 2006, Finer et al. 2006, Dempsey et al. 2009, Ewer et al. 2003, Greenough et al. 2002, De Zegher et al. 1993, Filippi et al. 2007, Synnes et al. 2001, Peltoniemi et al. 2005, Hall et al. 2005). A number of these studies reported administering anti–hypotensive therapy to infants who had co–morbidities. In the absence of a randomised controlled trials it is difficult to tease out if the cause of mortality was secondary to the effects of low BP, anti–hypotensive therapy alone, or both or if it was secondary to the coexisting morbidity. It is worthwhile pointing out the mortality rates in the randomised trial by Batton and colleagues (Batton et al. 2012) who investigated BP management in preterm infants. Unfortunately this multi centre trial study stopped prematurely due to poor recruitment.

Among the 10 infants that were recruited, the only 2 (20%) infants who died were those that were randomised to the placebo arm of the trial. In our cohort, the most common cause of death was due to infections (33%) followed by necrotising enterocolitis (22%), both of which are well known causes of mortality in extremely preterm infants (Ohlin et al. 2015, Bacak et al. 2005, Samuels et al. 2017, Qian et al. 2017).

Cranial ultrasound outcomes

The pre–defined outcome of haemorrhagic parenchymal infarct occurred only in 2/60 infants and therefore meaningful comparisons could be not be made between the three arms. Normal cranial ultrasound scan findings were most often found in the Active arm. The composite outcomes of rates of Grade 2–4 periventricular haemorrhage were significantly different (p=0.008) with it being highest in the Moderate arm (n=6, (30%)), followed by the Permissive arm (n=1, (5%)) and the Active arm (n=0). The combined outcome of Grade 2–4 periventricular haemorrhage, periventricular leukomalacia or parenchymal cysts were also significantly different (p=0.014) with it being highest in the Moderate arm (n=6, (30%)) followed by the Permissive arm (n=2, (10%)) and the Active arm (n=0). Thus infants who were maintained on a mean BP greater than 30 mmHg exhibited significantly lesser cranial ultrasound abnormalities.

One plausible explanation for this difference in the cranial ultrasound outcomes is that BP in the Moderate and Permissive arm had more variance when compared to the Active arm. The variance in the Active arm was lower than the other two arms which would have resulted from BP being actively supported to maintain it greater than 30 mmHg. Variance in BP is known to be associated with intraventricular haemorrhage (Cunningham et al. 1999, D'Souza et al. 1995). The finding of cranial ultrasound abnormalities in in-

fants whose mean BP was not maintained greater than 30 mmHg is consistent with the result of earlier studies examining this area (Miall-Allen et al. 1987, Watkins et al. 1989, Bada et al. 1990, Low et al. 1992, 1993, O'Shea et al. 1998). Miall–Allen (Miall-Allen et al. 1987) who studied 33 infants below 31 weeks of gestation using invasively recorded BP. They found that none of the infants had cranial ultrasound abnormalities when the mean BP was maintained greater than 30 mmHg. Watkins and colleagues (Watkins et al. 1989) investigated directly measured hourly BP in 131 very low birth weight infants and found that hypotension was directly related to the incidence of IVH. Bada et al (Bada et al. 1990) studied 100 infants in the first 48 hours with minute to minute continuously measured BP to find that infants with grade 2–4 intraventricular haemorrhage had consistently low BP. Low and colleagues (Low et al. 1992, 1993) examined 130 infants born at less than 34 week gestation. They examined continuously measured BP in the first 4 days of life and found that incidence of echosonographically demonstrable cerebral lesions at six months of age was higher in infants who were hypotensive and hypoxemic. Further work by O'Shea and associates (O'Shea et al. 1998) investigated perinatal risk factors for cranial ultrasound abnormalities in 48 infants weighing <1500 grams. They concluded that a systolic BP of < 33 mmHg in the first 12 hours was associated with an OR (95% CI) of 8 (2 to 31.3) of having intracranial abnormalities. More recently Faust and colleagues (Faust et al. 2015) examined a large cohort of nearly 5,000 premature infants born < 32 weeks gestation to identify association between hypotension and short term outcomes. They found that infants with hypotension (using different definitions) had a increased risk of mortality (OR 1.9), IVH (OR 1.6) and BPD (OR 1.34).

On the other hand, several studies failed to show an association between low BP and intraventricular haemorrhage (Trounce et al. 1988, D'Souza et al. 1995, Perlman et al. 1996,

Wiswell et al. 1996, Dammann et al. 2002, Limperopoulos et al. 2007, Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Engelke and Leviton 2011, Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Kuban, Paneth and Leviton 2011). Trounce and colleagues (Trounce et al. 1988) investigating clinical risk factors for periventricular leukomalacia in 200 infants weighing < 1501 grams found a strong correlation between immaturity and periventricular haemorrhage. They found hypotension (using systolic BP) was not predictive of the development of periventricular leukomalacia. D'Souza et al (D'Souza et al. 1995) examined associations with periventricular haemorrhage in 34 infants (gestational age 24 to 33 weeks). Invasive mean arterial BP was recorded every 15 minutes for the first 10 days and cranial ultrasound scans performed daily. They found that median BP was not different in infants who had periventricular haemorrhage and those without periventricular haemorrhage. They however noted a higher coefficient of variation in infants on the day of periventricular haemorrhage. The 15 minute recording of BP could have missed potential fluctuations in BP and could explain why there was no difference in BP between infants who had periventricular haemorrhage and those that had normal cranial scans. A case control study by Perlman (Perlman et al. 1996) examining risk factors for bilateral cystic periventricular leukomalcia from a large cohort of 632 infants weighing less than 1750 grams found an association with systemic hypotension which was absent after adjusting for confounders. One of the limitations of this work is the lack of description of how BP was measured. Wiswell and colleagues (Wiswell et al. 1996) investigated the effects of hypocarbia on the development of cystic periventricular leukomalacia. It is worth noting that in this cohort, there were nine infants who had significantly low BP and died before 21 days of age. Dammann (Dammann et al. 2002) examining systemic hypotension and white matter changes in 1607 infants found hypotension to be associated with cranial ultrasound abnormalities but it did not persist

after adjusting for potential confounders. Limperopoulos and colleagues (Limperopoulos et al. 2007) examined the relationship between current definitions of hypotension and early abnormal cranial ultrasound findings in 84 infants who were less than 30 weeks gestation with invasive arterial lines. 5–minute epochs of BP were recorded for up to 12 hours for 3 days and examined for associations with abnormal cranial ultrasound between day 5 and 10. The three common definitions used were; mean BP < 30 mmHg, mean BP < infants gestational age and mean BP < 10th percentile. None of these predicted cranial ultrasound abnormalities. A few reasons could explain a lack of association in this study. Firstly, BP was only recorded for 12 hours for 3 days. This would not be robust in reflecting changes in BP when compared to continuously measuring BP for 3 days. Secondly, this cohort of infants had a higher severity of illness as indicated by the SNAP score, hence there may other clinical factors which may cause cranial ultrasound abnormalities. More recent work by Logan and colleagues (Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Engelke and Leviton 2011, Logan, O'Shea, Allred, Laughon, Bose, Dammann, Batton, Kuban, Paneth and Leviton 2011) evaluated the relation between early postnatal hypotension and cranial ultrasound indicators of white matter damage imaged at nursery and at 2 years. This retrospective study examined 1041 infants with a gestational age less than 28 weeks. The method of BP used was not known to the researchers. This study used 3 indicators for hypotension such as lowest mean arterial BP in lowest quartile for gestational age, treatment for hypotension with vasopressors and BP lability. They found no association with any of the three indicators of hypotension and white matter damage. Though this study involved large number of infants, researchers did not have information on method of recording BP which is a major limitation of this study. These infants were followed up and underwent developmental assessment at 2 year of age. This study found none of the indicators for hypotension to be associated with Mental Development Index <

70 or Psychomotor Development Index < 70 after adjusting for potential confounders.

On performing further analysis on infants with cranial ultrasound abnormalities, we found that these infants had significantly lower mean BP, gestation and birth weight when compared to infants who had normal cranial ultrasound. Gestation being an important determinant of intracranial pathology is well established. Gestation was the only factor which remained significant after adjusting for several other factors including BP among this cohort. This could be explained perhaps by the lower number of infants (n=16) analysed in this subgroup. However, when dividing the cohort based on BP intervention levels, there was a significant difference in the incidence of intracranial pathology as explained above.

3.11 Post hoc analysis of combined blood pressure data

3.11.1 Analysis of blood pressure variability

There was no significant difference in the pre–specified clinical outcomes between study arms. The differences noted in BP between the three groups were only found at specific time points and could be related to the differential use of inotropes in response to different intervention levels. Therefore, it was reasonable to relate BP levels and variability in the combined dataset to periventricular haemorrhage and inotrope usage. Standard deviation and the coefficient of variation were calculated to represent BP variability.

Standard deviation

The standard deviation (SD) of BP were derived at 12–hourly BP epochs for the first 48 hours of life using 10–second continuously downloaded BP data. The 48–hour mark was chosen as it represented the period with the maximum BP data. Data from all infants in the study were considered for this analysis.

The mean SD (SD) at 0–12 hours, 13–24 hours, 25–36 hours and 37–48 hours were 2.7 (1.6), 3.1 (1.6), 2.4 (1.3) and 2.6 (1.8) mmHg respectively and not significantly different between time epochs. Though there was a change in the SD for all infants between the first 12 hours and the subsequent 12 hours, infants with grade 4 IVH were noted to have the largest change in SD between 12 and 24 hours (Figure 3.50).

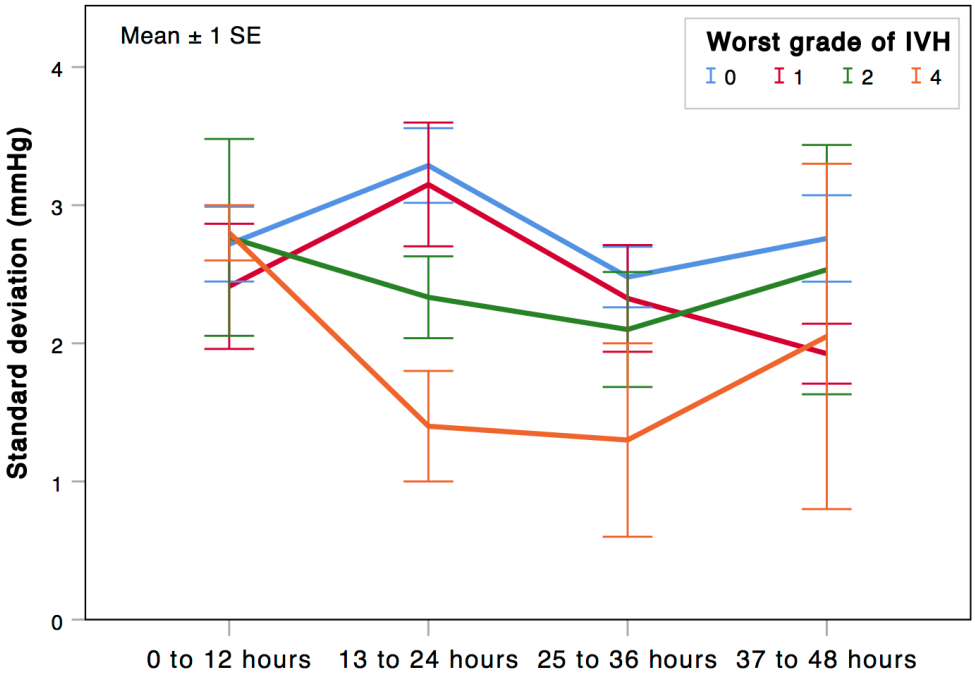


Figure 3.50: Standard deviation for the different BP epochs related to the different grades of IVH in the first week of postnatal life. Error bar represents ±1SE.

In order to explore the effects of change in SD, further analysis was performed by examining the difference in SD in each 24 hour period and how it relates to the degree of IVH found in cranial ultrasound scans performed in the first week of postnatal life. The change in SD was also related to the composite outcome of high grade IVH and white matter changes on cranial ultrasound scans.

There were no significant differences in the SD between the different grades of IVH. The

differences in SD for infants with grade 4 IVH were the largest with a positive trend in the first 24 hours (Figure 3.51) and a negative trend in the subsequent 24 hours (Figure 3.52). There were no significant differences in the composite outcomes of high grade IVH and white matter change on cranial ultrasound scans with BP SD at 24 hours (Figure 3.53) or 48 hours (Figure 3.54).

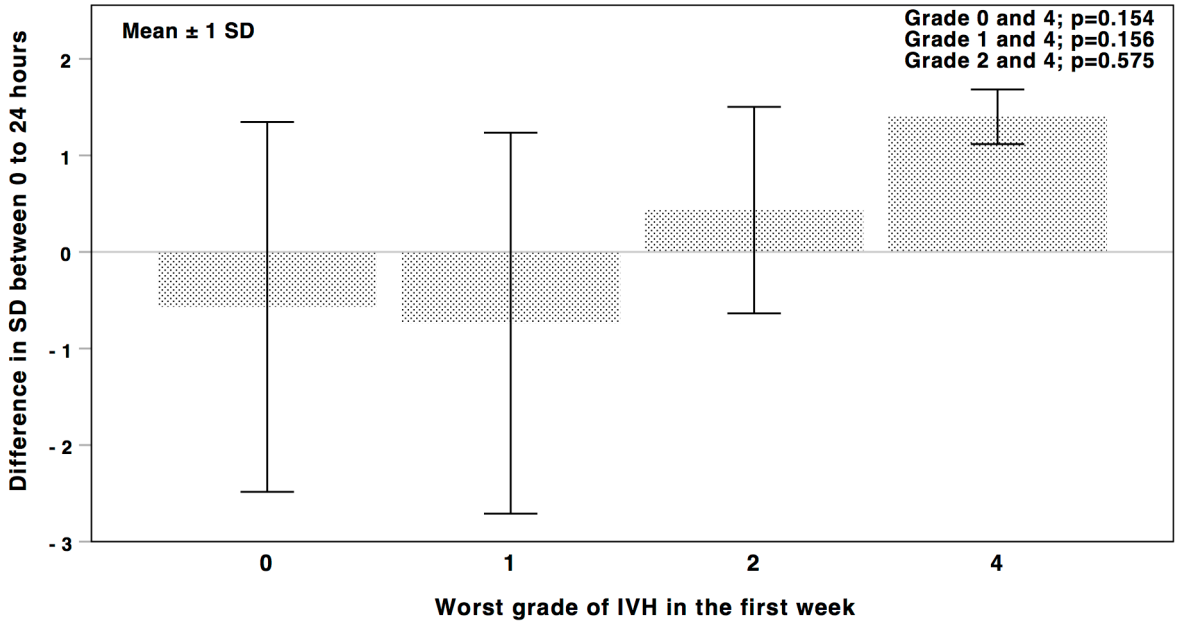


Figure 3.51: Difference in standard deviation in the first 24 hours related to the different grades of IVH in the first week of postnatal life. Error bar represents ±1SD.

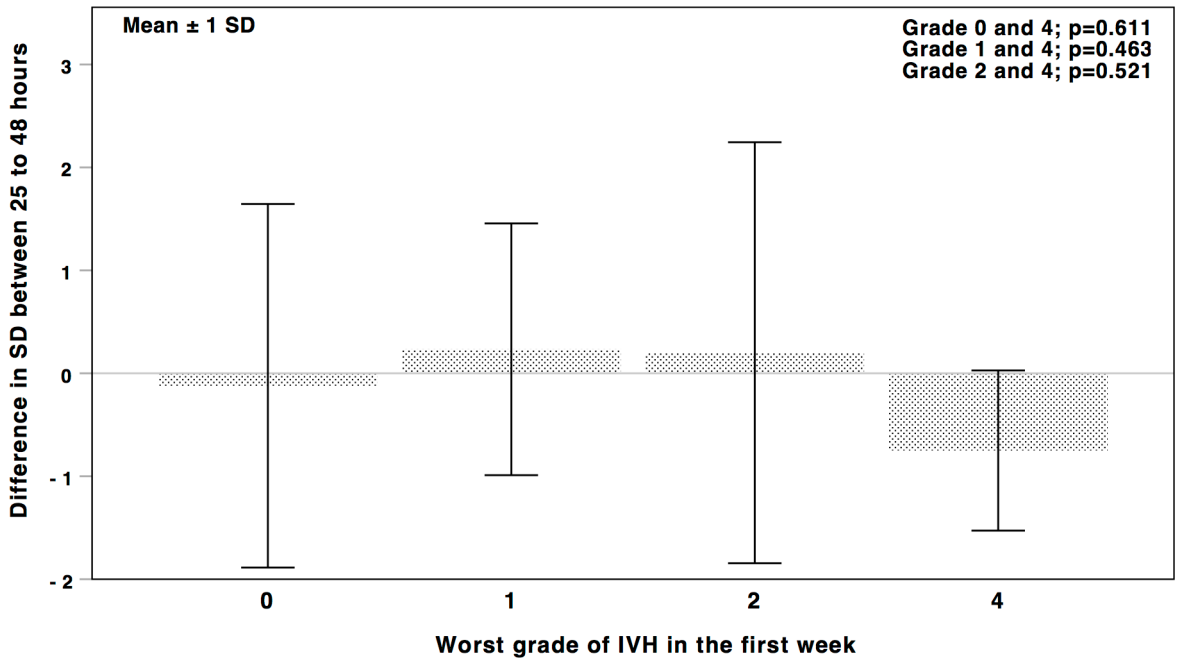


Figure 3.52: Difference in standard deviation between 25 to 48 hours related to the different grades of IVH in the first week of postnatal life. Error bar represents ±1SD.

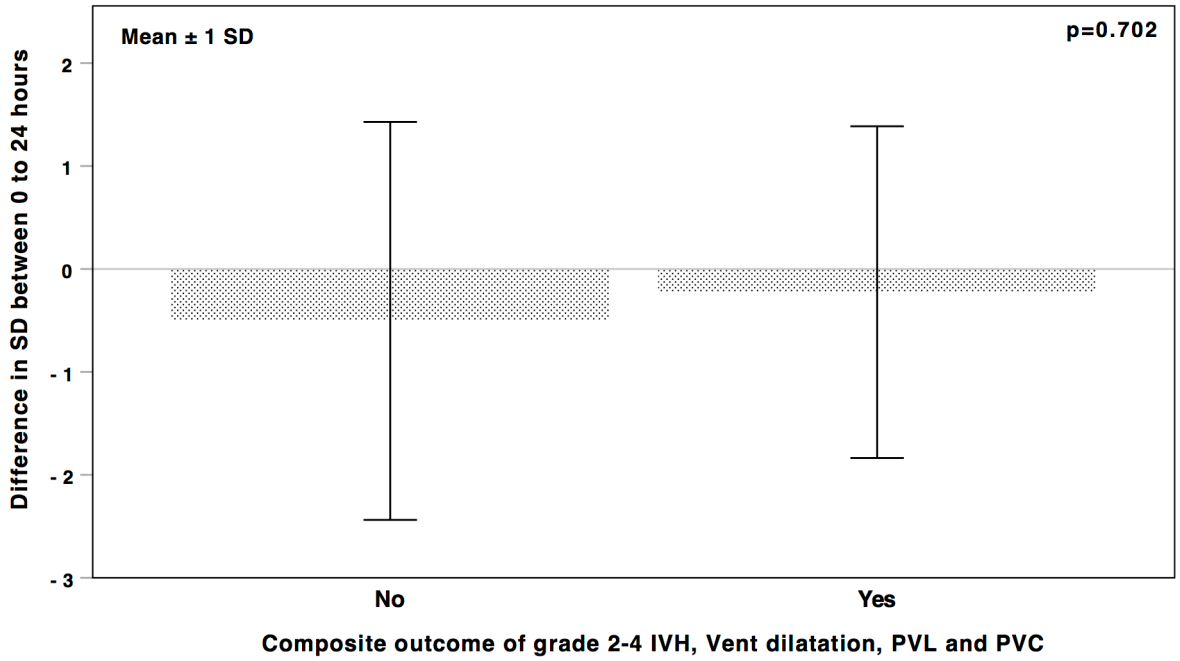


Figure 3.53: Difference in standard deviation in the first 24 hours related to the composite outcome of grade 2–4 IVH, ventricular dilatation, periventricular leukomalacia and porencephalic cysts. Error bar represents ±1SD.

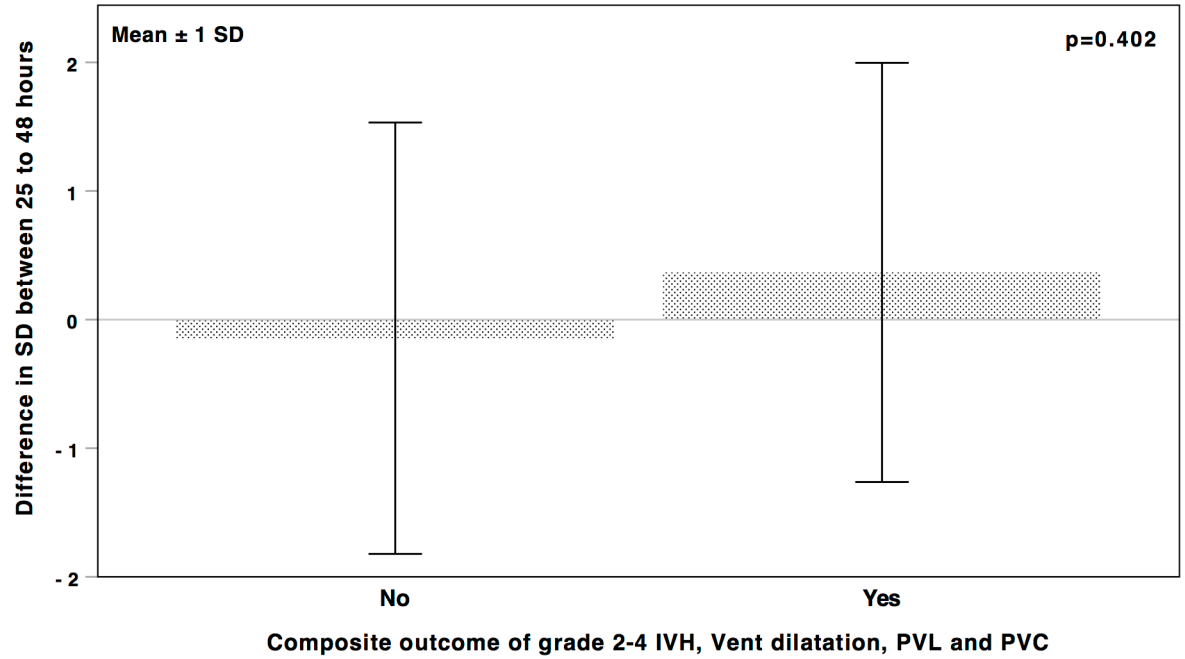


Figure 3.54: Difference in standard deviation between 25 to 48 hours related to the composite outcome of grade 2–4 IVH, ventricular dilatation, periventricular leukomalacia and porencephalic cysts. Error bar represents ±1SD.

Coefficient of variation

The coefficient of variation was examined as another measure of BP variability for the first 48 hours using 12–hourly epochs. The 48–hour mark was chosen as this period had maximum BP data. The coefficient of variation (CV) was calculated using the formula; (SD/Mean)*100 and was expressed as a percentage. The CV was calculated using the continuously downloaded 10–second data. The coefficient of variation at 24 and 48 hours were related to the worst grade of IVH during the first week of postnatal life.

The mean CV (SD) at 0–12 hours, 13–24 hours, 25–36 hours and 37–48 hours were 7.3 (4.8), 9.1 (4.4), 6.7 (3.5) and 6.5 (3.6) % respectively and not significantly different. The CV for infants with grade 4 IVH were noted to have the largest change especially in the first 24 hours but not significantly different when compared to the other groups (Figure 3.55).

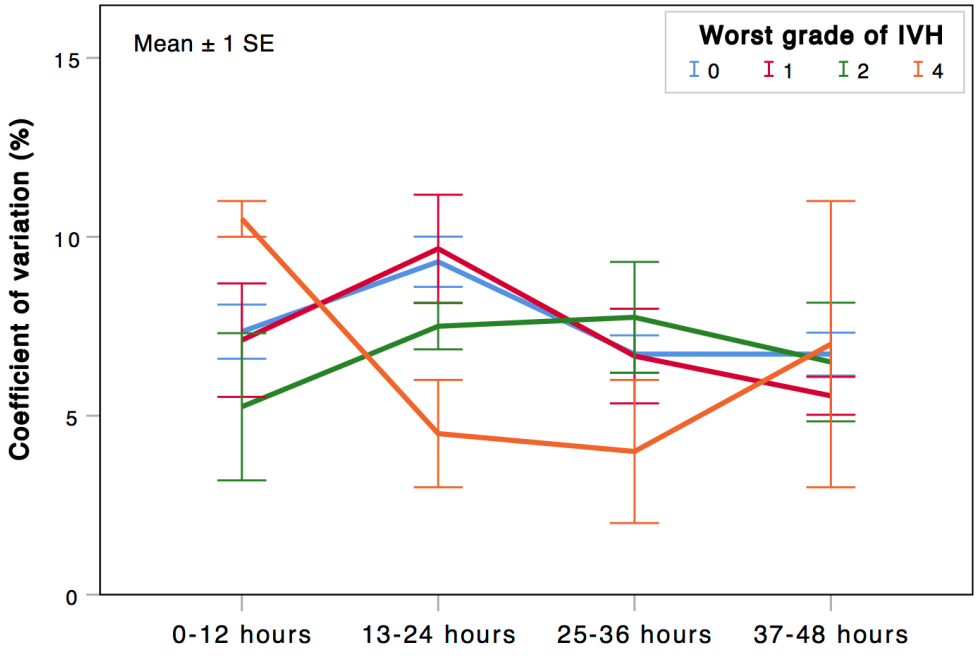


Figure 3.55: Coefficient of variation for the different BP epochs related to the different grades of IVH in the first week of postnatal life. Error bar represents ±1SE.

CV was related to inotrope usage in the first 48 hours of postnatal life. This showed

that infants who received inotropes in the first 12 hours had a significantly higher CV when compared to infants who did not receive inotropes (8.3 vs 5.3 %, p=0.04) with no difference in BP (31 vs 33 mmHg, p=0.21). There was no significant difference in the CV between infants who received inotropes and those who did not receive inotropes after 12 hours (Figure 3.56).

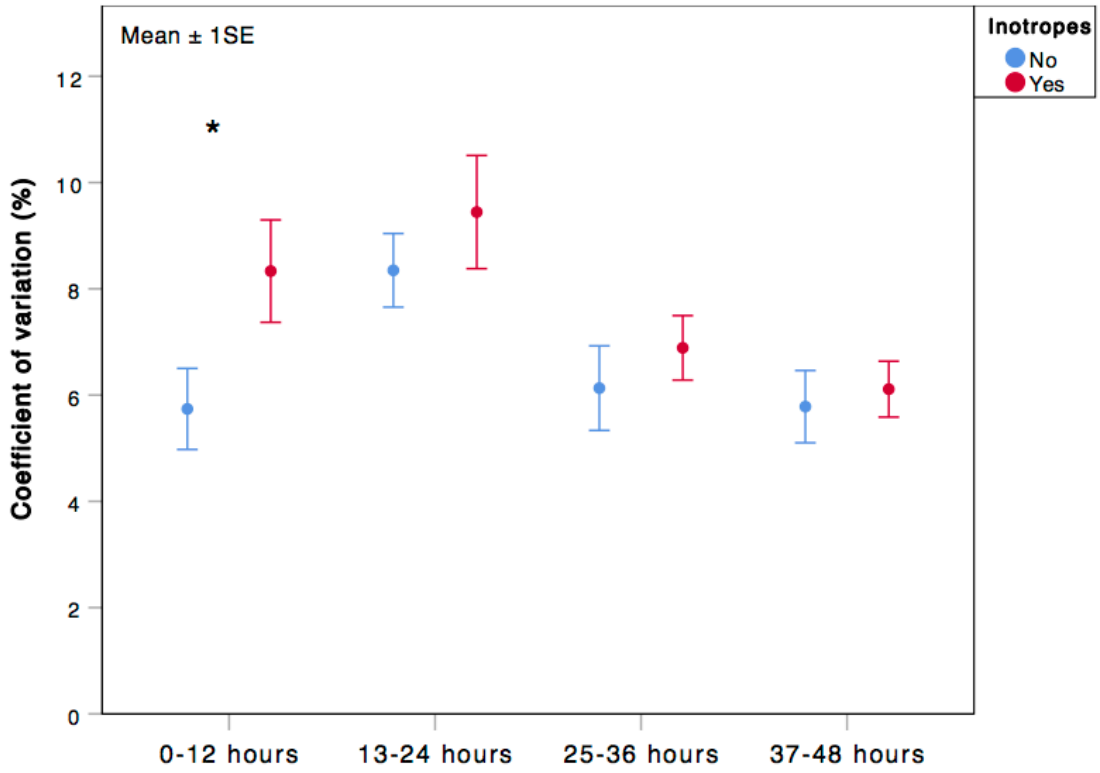


Figure 3.56: Coefficient of variation for the different BP epochs related to inotrope use in the first 48 hours of postnatal life. Error bar represents ±1SE. *p<0.05.

The difference in CV in the first 24 showed a positive, non–significant trend in infants with grade 4 IVH. It was different when compared to infants with lesser grades of IVH or no IVH (Figure 3.57). Though the difference for all groups were in the opposite direction in the subsequent 24 hours, infants with grade 4 IVH demonstrated the largest non–significant difference (Figure 3.58). The coefficient of variation was not significantly related to the composite outcome of high grade IVH and white matter damage on cranial ultrasound scans at in the first 24 hours (Figure 3.59) or the subsequent 24 hours Figure 3.60).

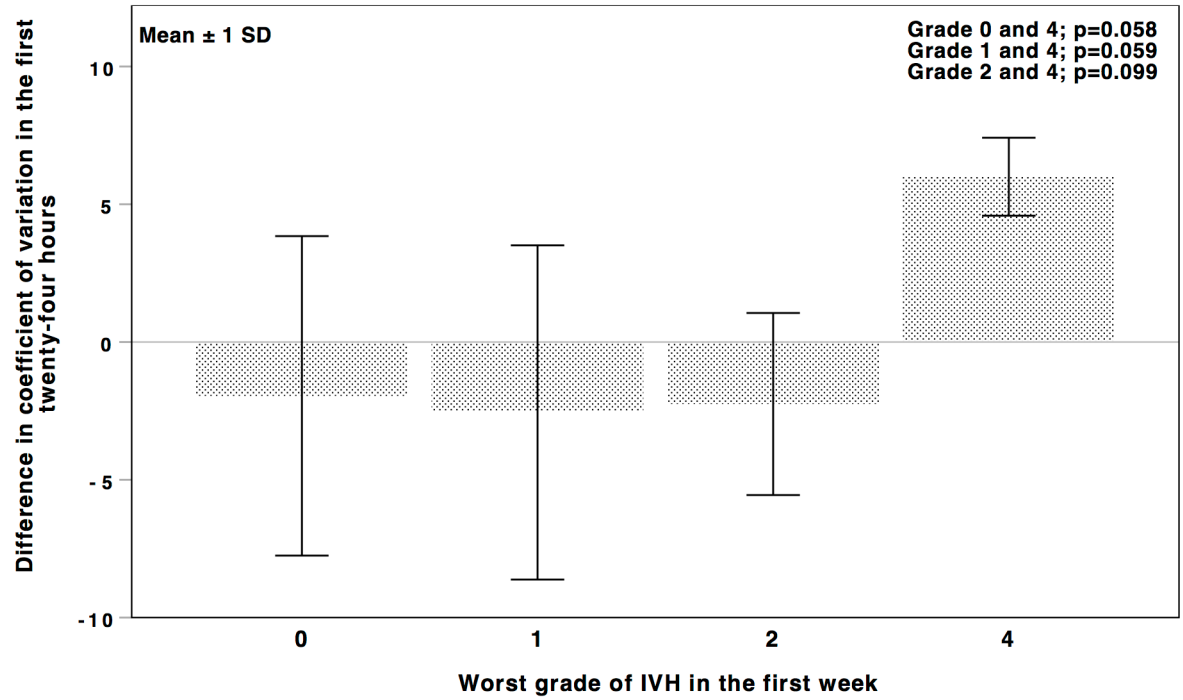


Figure 3.57: Difference in the coefficient of variation in the first 24 hours related to the different grades of IVH in the first week of postnatal life. Error bar represents ±1SD.

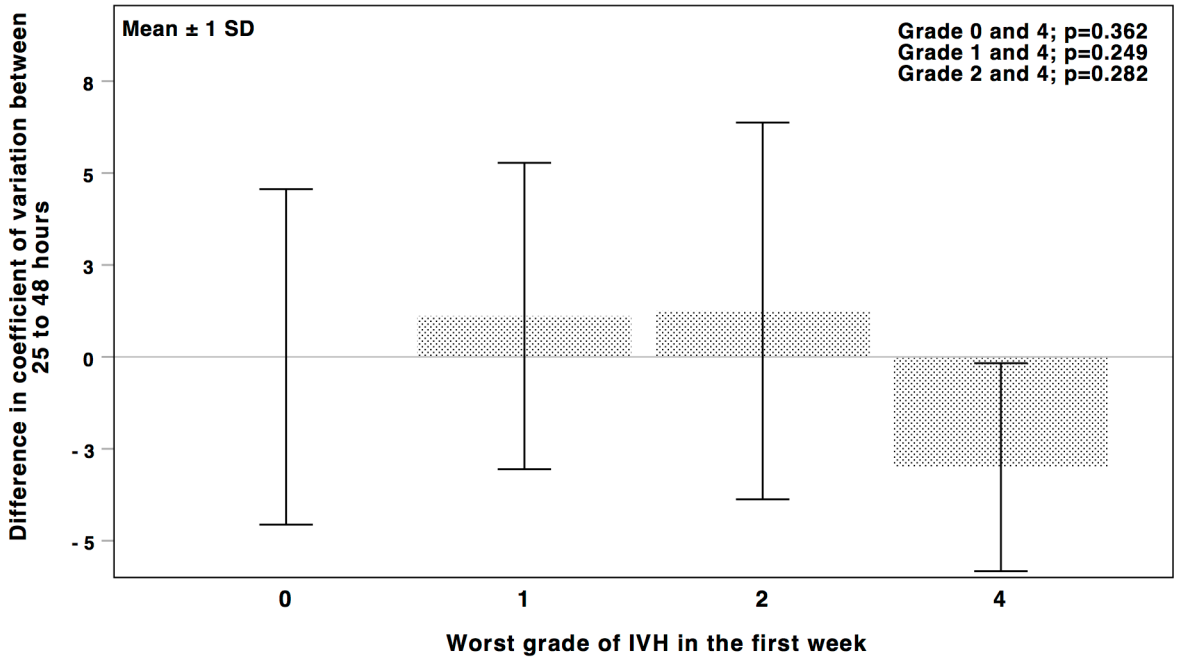


Figure 3.58: Difference in the coefficient of variation between 25 to 48 hours related to the different grades of IVH in the first week of postnatal life. Error bar represents ±1SD.

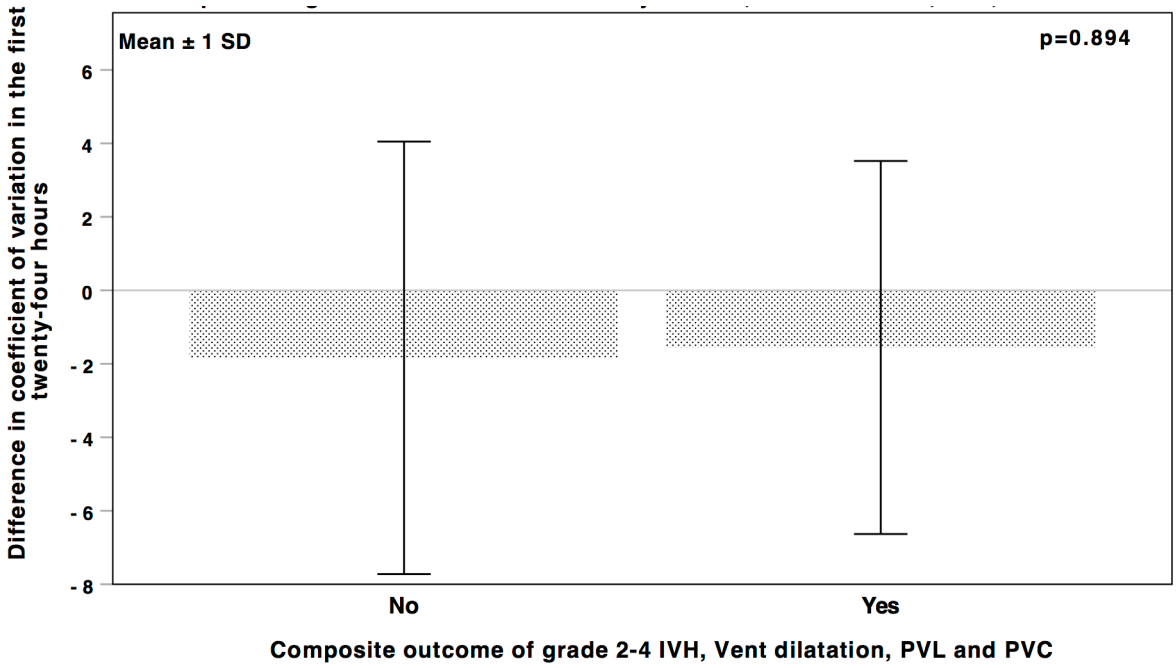


Figure 3.59: Difference in the coefficient of variation in the first 24 hours related to the composite outcome of grade 2–4 IVH, ventricular dilatation, periventricular leukomalacia and porencephalic cysts. Error bar represents ±1SD.

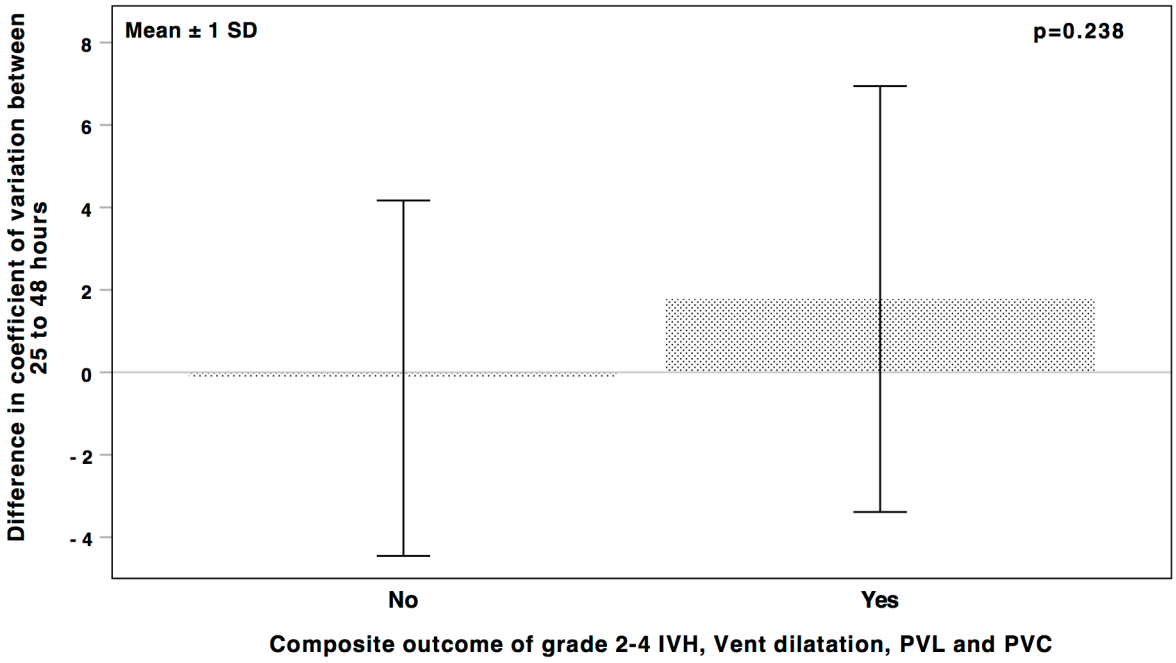


Figure 3.60: Difference in the coefficient of variation between 25 to 48 hours related to the composite outcome of grade 2–4 IVH, ventricular dilatation, periventricular leukomalacia and porencephalic cysts. Error bar represents ±1SD.

3.11.2 Proportion of blood pressure less than 30 mmHg

The proportion of BP which was less than 30 mmHg in the first 48 hours was calculated and expressed as a percentage for all infants in the study. This percentage was related to the grade of IVH in the first week of postnatal life.

The mean (SD) for the whole group was 19% (17%). The mean (SD) percentage of BP < 30 mmHg for infants without IVH was 16% (17%) and for those infants with grade 4 IVH was 46% (7%) and significantly different (p=0.017). There was a decreasing trend in the percentage of infants with BP < 30 mmHg and lesser grades of IVH. Infants without IVH had the least proportion of BP < 30 mmHg (Figure 3.61). There was a higher proportion of BP < 30 mmHg that was associated with the composite outcome of high grade IVH and white matter changes but this did not achieve statistical significance (p=0.057) (Figure 3.62).

However, further analysis (section 3.9, cranial ultrasound findings) performed using binary logistic regression showed that gestation was the only factor which predicted periventricular haemorrhage after adjusting for gestation, birth weight, inotrope administration, $PaCO_2$ and mean BP (8 to 72 hours).

Further BP analysis examining the proportion of BP < gestational age for the first 48 hours of postnatal life showed no significant difference between the different grades of IVH (Figure 3.63).

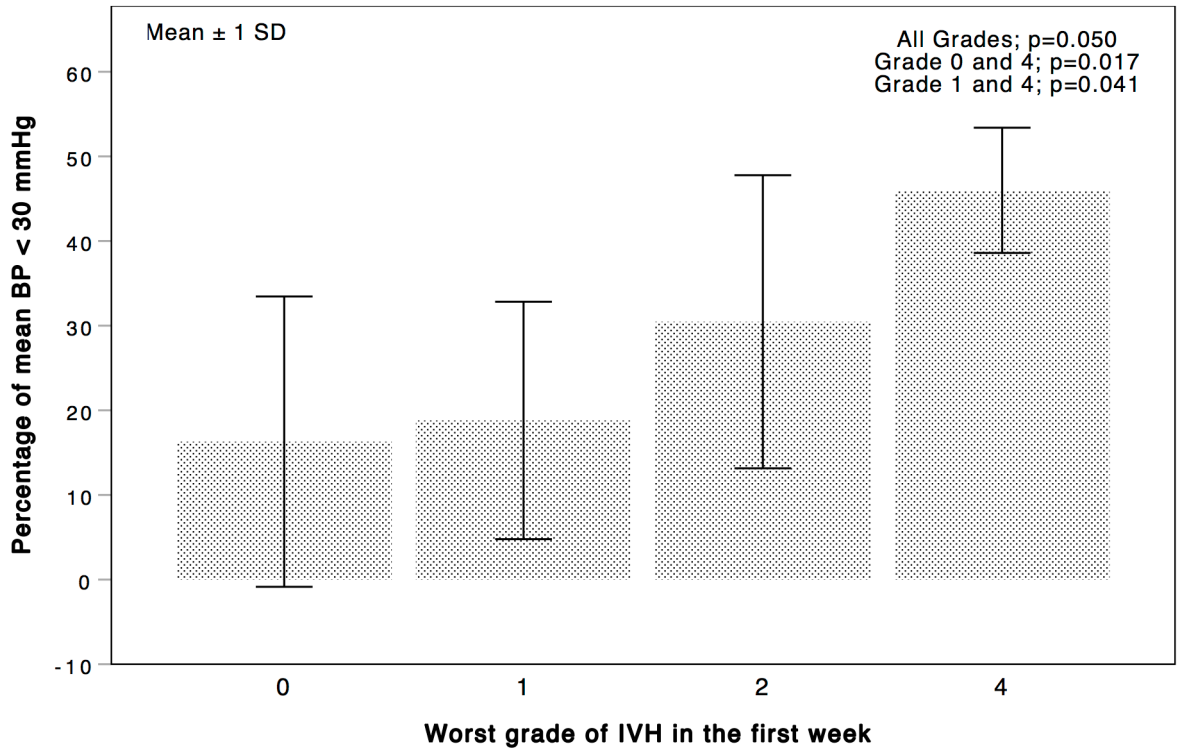


Figure 3.61: Proportion of BP which was < 30 mmHg in the first 48 hours related to the grades of IVH in the first week of postnatal life. Error bar represents ±1SD.

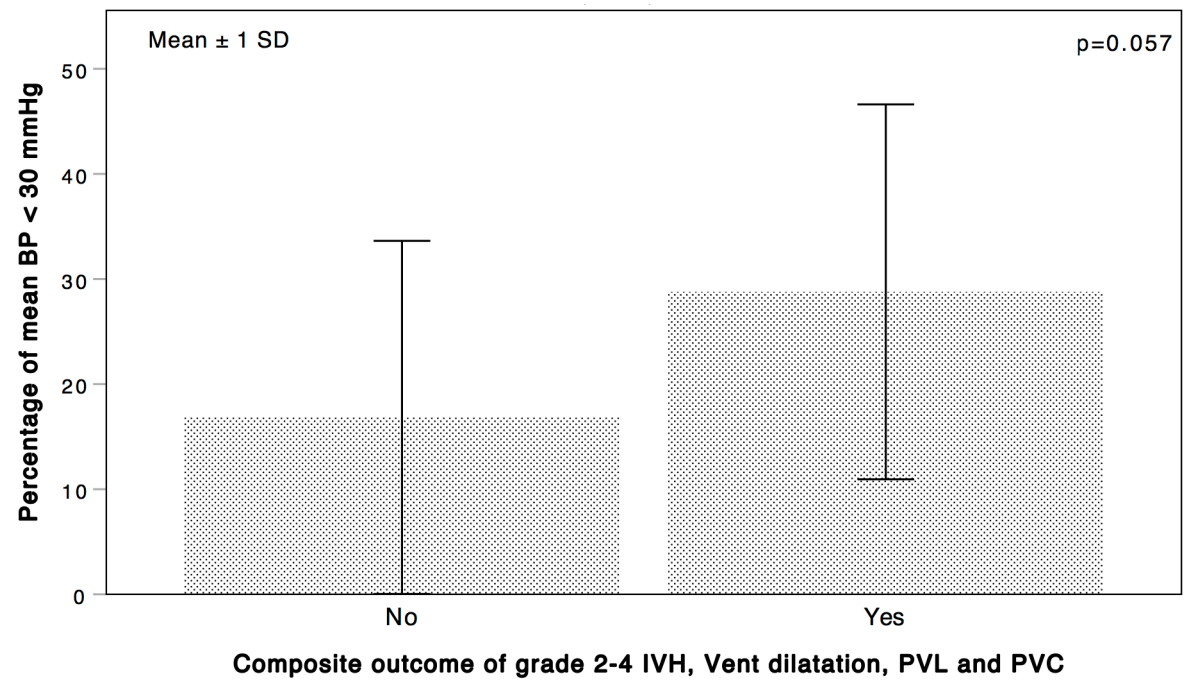


Figure 3.62: Proportion of BP which was < 30 mmHg related to the composite outcomes of grade 2-4 IVH, ventricular dilatation, periventricular leukomalacia and porencephalic cysts. Error bar represents ±1SD.

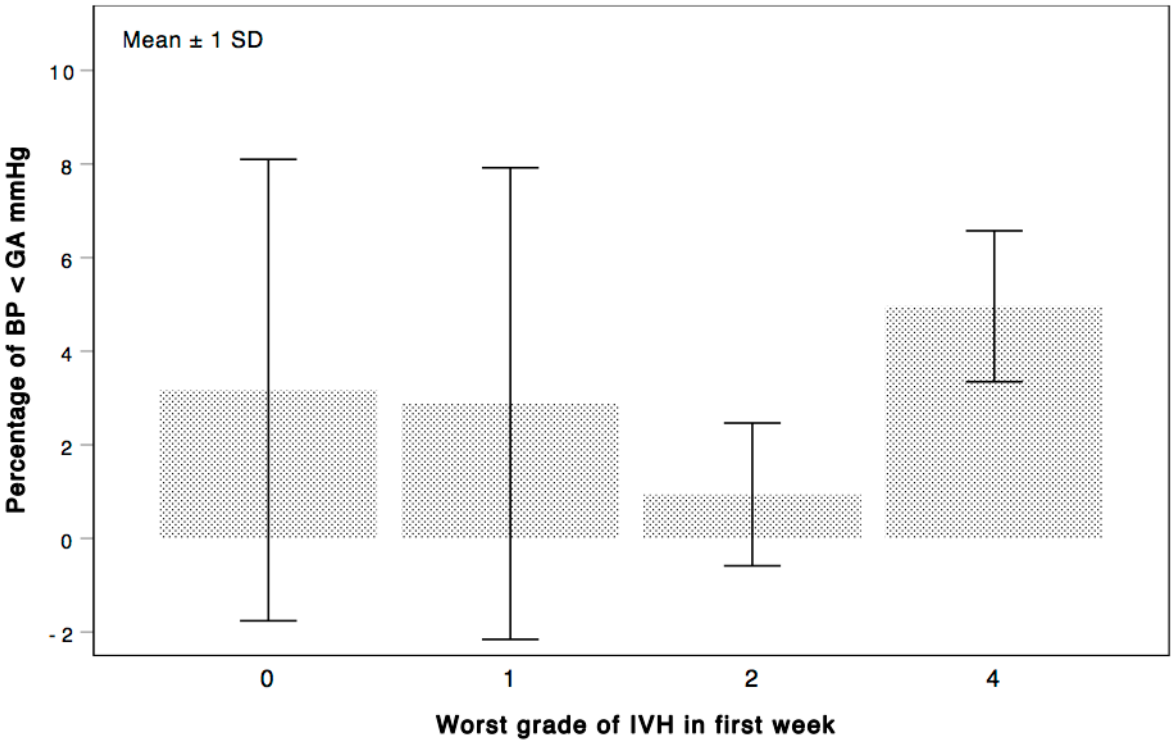


Figure 3.63: Proportion of BP which was < GA related to the grades of IVH in the first week of postnatal life. Error bar represents ±1SD.

3.12 Post hoc analysis of combined blood pressure data - discussion

A high variability in BP reflects a labile BP with fluctuations. Post hoc analysis of BP variability showed a large standard deviation and coefficient of variation with a changes in trend especially for infants who developed grade 4 IVH. In the first 12 hours of postnatal life, infants receiving inotropes were found to have higher BP variability (but no significant difference in BP) when compared to infants who did not receive inotropes. The finding of high BP variability associated with high grades of IVH is in agreement with others (Cunningham et al. 1999, D'Souza et al. 1995). The likely underlying mechanism being an ischaemia–reperfusion injury. This also supports the finding in this randomised controlled trial where infants in the Active arm had a more 'stable', less fluctuant BP resulting in the least cranial ultrasound abnormalities in this arm when compared to the other two arms. When relating these findings into clinical practice, fluctuations in BP especially

in the first 72 hours of postnatal life when intraventricular haemorrhage is most likely to occur should be avoided. Some of the practical steps clinicians can take to reduce these fluctuations in BP include avoiding generous fluid boluses and ensuring an appropriately diluted inotropic solution is used which will prevent erratic surges in BP in this vulnerable group of infants.

Infants with a higher proportion of BP < 30 mmHg were found to have significantly higher rates of grade 4 IVH which is in keeping with the findings from previous studies (O'Shea et al. 1998, Low et al. 1993, 1992, Bada et al. 1990, Watkins et al. 1989, Miall-Allen et al. 1987). In this study, infants who had high grade IVH were of a lower gestational age. Gestation was found to be the only predictor for grade 4 IVH in this cohort of infants. Studies have shown that infants with mean BP maintained around 30 mmHg are likely to have improved cerebral perfusion (Munro et al. 2004) and therefore less likely to be susceptible to the risk of ischaemia–reperfusion injury which could result in intracerebral haemorrhage and adverse neurodevelopmental outcomes.

3.13 Supplementary work

3.13.1 Variation between staff recorded and continuously download invasive blood pressure in extremely premature newborn infants

The methods used in this work has been described in section 2.12.1

Results

A total of 1180 measurements were compared over the first week of life in 42 extremely preterm infants. The mean (SD) gestation and birth weight was 25.7 (1.4) weeks and 802 (177) grams respectively. The median (IQR) BP recorded by staff was 33 (30–36) mmHg compared to electronic monitor recording of 33.5 (30.4–36.5) mmHg for this cohort. Some of the greatest differences between continuous and staff recorded measurements were seen in patients receiving inotropic support. Spearman's rank correlation showed a strong correlation between the two measurement methods ($r = 0.807$, $p < 0.001$) (Figure 3.64). The mean (SD, 95%CI) bias was -0.11 (3.17, -0.29 to 0.07) mmHg. The lower LoA (95% CI) being -6.33 (-6.64 to -6.01) mmHg and upper LoA (95% CI) being 6.11 (5.79 to 6.42) mmHg (Figure 3.65). There was no evidence of proportional bias.

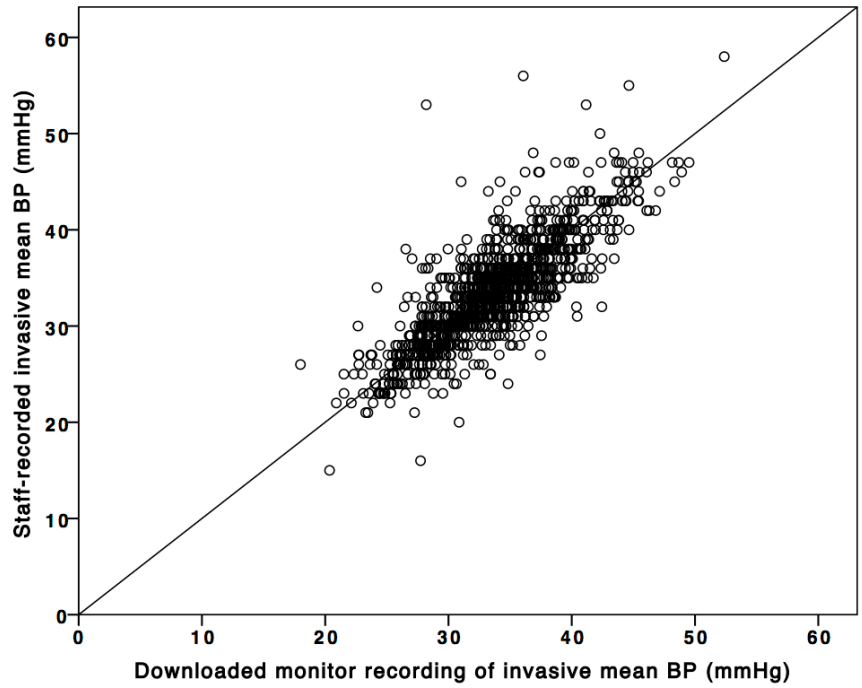


Figure 3.64: Scatter plot illustrating the relation between staff-recorded and downloaded monitor BP along with the line of equality.

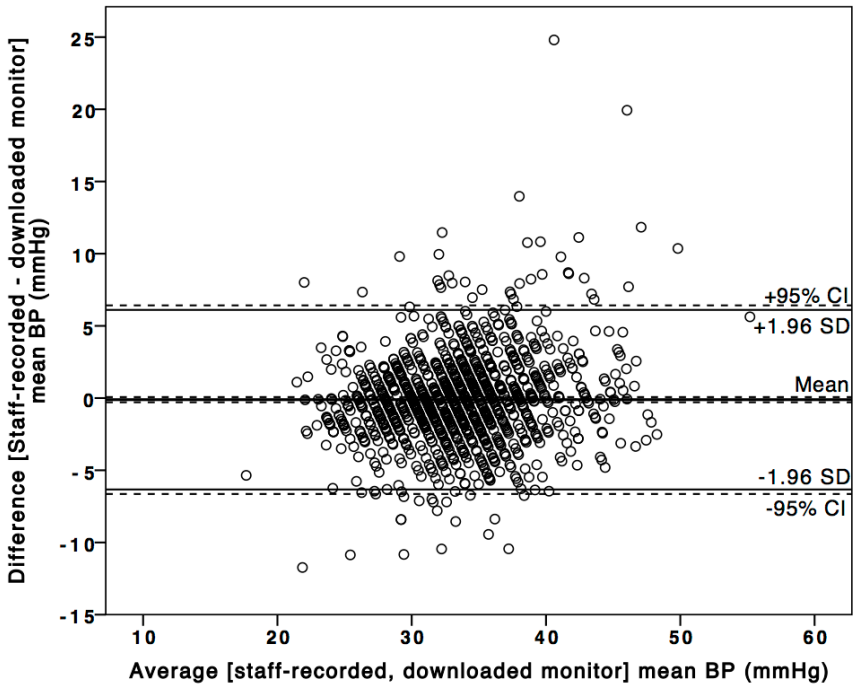


Figure 3.65: Bland-Altman plot illustrating the mean of the difference and the 95% limits of agreement.

3.13.2 Variation between staff recorded and continuously download invasive blood pressure in extremely premature newborn infants - discussion

This study comparing staff recorded BP with continuously downloaded BP showed that 95% limits of agreement to be between approximately ± 6 mmHg with no evidence of systematic bias. This is a significant difference especially when the clinician wants to maintain the BP above a particular range. The main source of this variation can include periods of high BP, which would have occurred after commencing inotropic support. Once the target BP has been achieved, the dose of inotropes may have been adjusted to reduce BP to the desired levels. The staff recording this BP and the clinician who relies only on the nursing chart may not be aware of the high levels of BP unless the minute-by-minute trend rather than hourly trend in BP is examined.

These differences between staff recorded BP and the more reliable continuous invasive BP measurements may add statistical noise to studies examining the association between BP and outcome. Inotropes may have been started for hypotension which was present on continuous monitoring, but missed on hourly measurements. Although this problem may be overcome in large studies, we would urge some caution in interpreting studies using staff-recorded BP. The findings of this study are similar to previous work reported by Hug and colleagues (Hug et al. 2011) who compared clinician recorded invasive BP measurements against automated archiving methods from the same invasive monitor in an adult intensive care setting.

The strength of this study include the large number of invasive BP data points that was averaged to obtain the monitor BP in the majority of infants make the data quite robust.

The study population is fairly well distributed with a sufficiently large number of infants from different gestations being included in this study. This study compared data obtained from several staff during day and night time. We did not compare recordings taken by the same staff for infants of different gestation nor did we look at the difference in day and night staff recording the data. The association between treatment with inotropes and adverse outcome must be examined to determine whether this is due to the drugs themselves, or the underlying cardiovascular disturbance being treated. Inotrope treatment may be a more sensitive marker of haemodynamic disturbance, as it overcomes sampling and measurement methods in documenting hypotension, and inotropes may also be given for low cardiac output states which are not associated with hypotension.

3.13.3 Comparison between invasive and non–invasive blood pressure measurements

The methods used in this work has been described in section 2.12.2

Results

The mean (SD, range) gestation and birthweight of the group was 26.4 (4, 23–38) weeks and 972.1 (826, 600–3560) grams. BP measurements for each infant varied between 6 to 12 BP measurements. There were 72 paired measurements between invasive mean arterial BP and mean cuff BP measured from hand and 59 paired measurements between invasive mean arterial BP and mean cuff BP measured from the leg (Appendix, Table 7.3).

Invasive arterial blood pressure and cuff blood pressure measurements from the arm

The average (SD) mean invasive arterial BP was 32.7 (5.4) mmHg and the average (SD)

mean BP measurements was 43.4 (6.6) mmHg. The average (SD, 95% CI) mean difference [UAC-cuff arm] was -10.9 (8.7, -12.9 to -8.9) mmHg. The lower LoA (95% CI) were -27.9 (-31.4 to -24.4) mmHg and the upper LoA (95% CI) were 6.1 (2.6 to 9.6) mmHg (Figure 3.66). There was a significant difference between the two methods of measurement of BP (one sample t–test, $p<0.001$). The mean difference was significantly different from zero. There was no evidence of proportional bias.

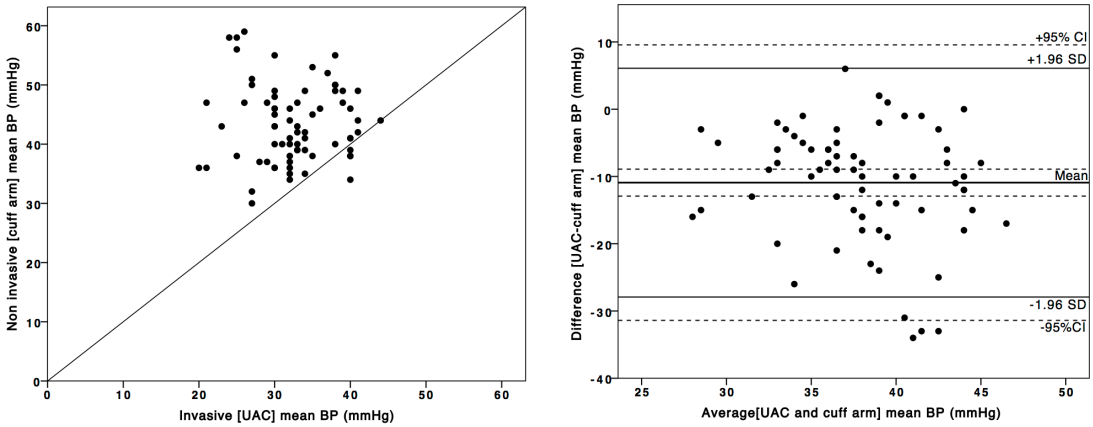


Figure 3.66: Scatter plot of the relation between invasive and non–invasive BP and Bland-Altman plot illustrating the mean of the difference and the 95% limits of agreement.

Invasive blood pressure and cuff blood pressure measurements from the leg

The average (SD) mean invasive arterial BP was 32.7 (5.4) mmHg and the average (SD) mean BP measurements was 40.6 (8.4) mmHg.The average (SD, 95% CI) mean difference [UAC-cuff leg] -6.1 (7.9, -8.2 to -4.1) mmHg. The lower LoA (95% CI) were -21.8 (-25.3 to -18.2) mmHg and the upper LoA (95% CI) were 9.5 (6.0 to 13.1) mmHg (Figure 3.67). There was a significant difference between the two methods of measurement of BP (one sample t–test, $p<0.001$). There mean difference was significantly different from zero. There was evidence of proportional bias, with invasive BP measurements reading higher at lower values and cuff BP measurements reading higher with higher BP measurements.

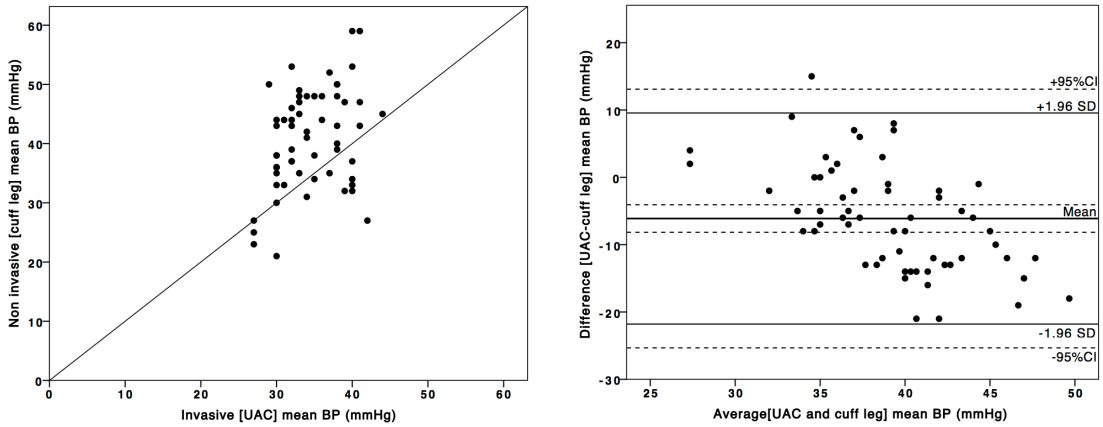


Figure 3.67: Scatter plot of the relation between invasive and non–invasive BP and Bland-Altman plot illustrating the mean of the difference and the 95% limits of agreement.

3.13.4 Comparison between invasive and non–invasive blood pressure measurements - discussion

This work examining a small number of infants who were randomly selected to have paired invasive and non–invasive BP showed that the mean difference between invasive BP and non–invasive BP measurement from the arm showed a mean difference of -10.9 mmHg and that from the leg showed a mean difference of -6.1 mmHg, both non–invasive BP measurements tending to overestimate when compared to the invasive BP. Our results were comparable to work done by Troy et al (Troy et al. 2009) who examined 38 infants with the majority of non–invasive BP measurements using Spacelabs SL1700 monitor system (Spacelabs Healthcare, Issaquah, WA, USA) being obtained from the leg to be 12 mmHg.

The strength of this study includes that a good number of non–invasive BP measurements were obtained from both the arms and legs. This work emphasises that clinicians need to be aware when using non–invasive BP to monitor cardiovascular status.

Invasive BP being the 'gold standard' should be used where available in clinical prac-

tice. Given this difference between invasive and non–invasive BP is clinically significant, staff should have a low threshold to use invasive BP to monitor BP and fine tune inotropic support.

Chapter 4

Discussion

4.1 Summary of results

4.1.1 Relationship between cerebral blood flow, cardiac output and blood pressure

Cerebral blood flow volumes measured in this group of infants were lower than those previously reported in more mature well infants who were breathing unaided (Sinha et al. 2006). Cerebral blood flow volumes increased significantly between day 1 and 3 of postnatal life in keeping with reports from other studies (Meek et al. 1998, Noone et al. 2003) that used different methods for measuring cerebral blood flow. Cerebral blood flow had a direct significant relationship with continuously downloaded invasive mean arterial BP and this relation was stronger in infants with a haemodynamically insignificant PDA.

Cardiac output increased significantly between day 1 and 3 to previously reported values (Kissack et al. 2005, M. Kluckow 1996, Pladys et al. 1999, Victor, Appleton, Beirne, Marson and Weindling 2006). Cardiac output was not significantly related to cerebral blood flow volumes, a finding replicated by others (Victor, Appleton, Beirne, Marson and Weindling 2006, Sirc et al. 2013). Cardiac output was not significantly associated with BP in this group of infants, a finding reported by others (Pladys et al. 1999). Finally, approximately 16% of cardiac output was directed to both common carotid arteries in agreement with with previous studies (Kandel et al. 2012, Sato et al. 2011, Sinha et al. 2006, Magistretti 1999).

4.1.2 Relationship between cerebral blood flow and blood gas parameters

Cerebral blood flow was found to have a non–significant relationship to blood $PaCO_2$ level unlike other studies (Pryds et al. 1990, Fenton et al. 1992, Levene et al. 1988) which

showed a direct relationship between these variables. The lack of a strong relationship could be due to a number of reasons. The narrow range of $PaCO_2$ levels maintained in infants in this study could be one of the reasons. Only five infants had $PaCO_2$ >7 KPa, the breakpoint at which Noori and colleagues (Noori et al. 2014) demonstrated that cerebral blood flow reactivity was only present when the $PaCO_2$ was >7 KPa. The relationship with cerebral blood flow and lactate levels were non–significant on both day 1 and 3. Other possible reasons for a lack of relationship include the lack of multiple measurements of cerebral blood flow which may not reveal the true relationship between these variables.

4.1.3 Relationship between circulatory parameter and markers of peripheral perfusion

The relationship between various circulatory parameters and markers of peripheral perfusion such as capillary refill time, lactate and urine output were complex and non–significant. Lactate levels were inversely related to cardiac output on day 1 only which was in agreement with previous work (Miletin et al. 2009). This lack of relationship could be due to drugs causing vasoconstriction of peripheral circulation, effect of PDA on the left ventricular output, poor peripheral vascular tone in infants of this gestation or due to the lack of multiple measurements which may not reflect the true relationship.

4.1.4 Relationship between electroencephalographic parameters physiological variables and blood pressure

All aEEG/EEG parameters measured were directly and significantly related to gestation, a well documented finding (Vesoulis et al. 2014, Zhang et al. 2011, Olischar et al. 2004, Hayakawa et al. 2001, Vecchierini et al. 2007, Hellstrom-Westas et al. 2006, Selton 2000). EEG parameters were found to be suppressed by acidosis and hypercarbia as demonstrated

by other studies (Granot et al. 2012, Wikstrom et al. 2011, Victor et al. 2005*a*, Eaton et al. 1994*b*). Discontinuity obtained in this study were comparable to other reports (Victor, Marson, Appleton, Beirne and Weindling 2006, Victor et al. 2005*b*) both on day 1 and 3. There were no significant relationship between EEG parameters and blood flow parameters such as cardiac output or cerebral blood flow measured using the right common carotid artery. Several reasons account for a lack of relationship including cardiac output being over-estimated using the left ventricular output, effect of PDA in reducing the systemic vascular resistance, effect of cerebral auto-regulation and lack of multiple/ continuous measurements. None of the aEEG/EEG variables examined were statistically significant between the three arms of the study.

This study found a strong correlation between continuously downloaded invasive mean arterial BP and various EEG variables. After correcting for clinical characteristics such as pH, $PaCO_2$, serum lactate, common carotid artery blood flow, left ventricular output, morphine and inotrope administration EEG remained significantly correlated with invasive BP during the two time points on day 1 and 3 of postnatal life. This was in contrast to findings from other studies (Shah et al. 2013) which failed to find a significant relationship between BP and EEG which could be due to the use of non–invasive BP.

4.1.5 Blood pressure measurements and inotropic support

The majority of infants (51/60, 85%) had high quality continuously downloaded BP data available between 12 and 48 hours of age. The rate of rise of BP was lower than those reported by others (Batton et al. 2014, 2007, Cordero et al. 2002) which is due to the use of a mixture of BP data obtained from retrospective review chart review. A significant separation was achieved in BP during the first 48 hours with it being highest in the Ac-

tive arm and lowest in the Permissive arm. There was no significant difference between the three arms beyond 72 hours of age. BP was most stable in infants in the Active arm when compared to the other two arms. This study has found that randomising infants to different BP treatment thresholds resulted in a significant separation in invasive mean BP in the first 48 hours of postnatal life.

Non–invasive BP was available in 9 (15%) infants. These infants were of a higher gestation when compared to those who had invasive BP measurements. When non–invasive BP measurements were taken into account, the period where BP was different between the three arms was reduced to only between 12–15 hours which is due to the over-estimation of BP when non–invasive data is included.

Randomising infants to different BP threshold also resulted in a significant difference in the rates of inotropic support between the three arms of the study with it being highest in the Active arm (79% received inotropic support) and least in the Permissive arm (48%). However, when gestation-wise inotropic requirement was examined, the majority of infants under 25 weeks received inotropic support where as the majority of infants above 27 weeks did not receive inotropic support irrespective of the arms that they were recruited to. Dopamine was the most frequently used inotropic agent used.

4.1.6 Variation between staff recorded and continuously downloaded invasive blood pressure in extremely premature newborn infants

This study compared staff recorded invasive mean BP and compared it to continuously downloaded BP data in extremely preterm infants and found the 95% limits of agreement

to be approximately ± 6 mmHg. Infants receiving inotropic support had the most variation in BP. The main source of this variation arises from the peaks of BP especially after commencing inotropic support which is then adjusted and secondly from clinician bias when picking BP values to be recorded in the charts. Clinicians should be aware of this especially when adjusting inotropic support.

4.1.7 Comparison of non–invasive blood pressure and invasive blood pressure

Non–invasive BP is known to over-estimate BP however, this study established the degree of difference between invasive and non–invasive BP using the current GE monitoring systems in the unit. We found that the mean difference (95% CI) between invasive BP and non–invasive BP measurement from the arm showed a mean difference of -10.9 (-12.9 to -8.9) mmHg and that from the leg showed a mean difference of -6.1 (-8.2 to -4.1) mmHg, both non–invasive BP measurements tending to overestimate when compared to the invasive BP. This degree of difference was comparable to other studies (Troy et al. 2009).

4.1.8 Clinical outcomes of the blood pressure intervention levels

This study examined multiple secondary outcomes. Being a pilot study, we wanted to investigate what outcomes will need to be explored in larger trials. Though this pilot study was not sufficiently powered to detect significant differences in clinical outcomes, we found no significant differences in the various system-wise clinical outcomes. We found a non–significant trend in serum creatinine levels and non–NEC, with it being highest in the Permissive arm and lowest in the Active arm. Despite a small sample, it was reassuring that there were no significant difference in mortality between the three arms of the study.

The pre–defined outcome of death and haemorrhagic parenchymal infarct was very low (only 2/60 infants). Infants with periventricular haemorrhage were of a significantly lower gestation and birth weight and also had a lower mean arterial BP when compared to infants who had a normal cranial ultrasound. The Active arm had infants with the most number of normal cranial ultrasound scans. The composite outcome (only on post–hoc analysis) of Grade 2–4 periventricular haemorrhage, periventricular leukomalacia or parenchymal cysts were significantly different with it being highest in the Moderate arm (n=6, (30%)) followed by the Permissive arm (n=2, (10%)) and the Active arm (n=0). As BP was most stable in the Active arm and more labile in the Moderate arm, we speculate that this may explain why the Active arm had the most number of infants with normal cranial ultrasound scans whereas the Moderate arm had the most number of infants with cranial ultrasound abnormalities. Though only found in post–hoc analysis, this result should be treated with caution at this stage and further larger trials are needed to explore this finding.

4.2 Limitations of the study

This pilot, feasibility trial was limited in the number of infants examined. As an exploratory study, several clinical outcomes were examined for which the study was not sufficiently powered. However, investigating several variables will give valuable information for the planning of larger trials. This study was not sufficiently powered to detect significant differences in major clinical outcomes. The other limitation of the study was with regards to the fixed time point measurement of the physiological variables in the first three days of life. An increase in the frequency of measurement of the various physiological variable would yield more information and may help us to understand the relationship between these variables and BP more accurately. The inherent variability of any method including the right common carotid artery blood flow volumes may mask the true relation-

ship between cerebral blood flow with other parameters. We examined only one aspect of the cardiovascular management which was BP intervention level. As this is only one factor which can influence cardiovascular management, there are lots of other factors including treatment for low flow states and treatment of PDA.

The Permissive arm in this study had a lower BP threshold of 19 mmHg before which inotropic treatment was instituted. This figure was chosen for pragmatic reasons and ensure that there was a wide enough separation in BP when compared to the infants of the lowest gestation recruited to the Moderate arm. However, this threshold was lower than the corresponding 10^{th} centile for each gestation.

aEEG/EEG activity analysis was restricted to a 2–hour period around which the physiological parameters were measured. A more detailed analysis of the aEEG/EEG activity at other times may yield valuable information on the relationship between aEEG/EEG activity and BP. Relating long term aEEG/EEG outcomes requires a large number of infants of adequate power to be examined which is outside the scope of this study.

Lastly, none of the infants had head circumference measured at birth which meant that results of cerebral blood flow could only be expressed as ml/kg/min rather than the widely used units of ml/100gms/min.

4.3 Strengths of the study

This is one of the largest study to report its findings. All of the planned sixty infants were recruited, randomised, underwent detailed measurements and studied with no protocol deviations or infants withdrawing from the study. There were a substantial number of ex-

tremely preterm infants on whom high quality data was captured. We gained considerable information on consenting infants to such trials from this work and also obtained valuable feedback from parents on why they refused to give consent to entry for such a study. This information would be valuable in planning future trials in this area.

The median age of recruitment of infants into the study was 8 hours, which meant a major proportion of the transitional circulation very early on the infant's life could be examined. Invasive BP data was available for 85% of infants in the study. This invasive BP data was downloaded every 10 seconds for the first week of life which gave valuable information on the trend of BP. This quality of data has made it useful to understand the variance in BP and also to correlate this with continuous electroencephalographic data which was available. In addition to BP and electroencephalographic data, physiological parameters were also studied in all infants twice in the first three days of life. All the infants across the three arms had the same treatment guideline present at the cot side, which made it easier for clinicians to follow. This uniformity in treatment eliminated treatment administered as a source of variation between the three groups.

This pilot study examined the three most frequently practiced BP intervention levels. This is in comparison to previous studies and other current studies which predominantly aim to examine only the Moderate and Permissive arms. Though the number of infants compared were small, this study has given us the confidence that it is feasible to carry out such studies. Though not powered to detect changes in mortality, it is reassuring to known that none of the deaths were directly related to BP intervention levels.

This study informed us on what the cerebral blood flow is for extremely preterm infant who

are ventilated in the first three days of life using the right common carotid artery, a method that has not been previously used for ventilated extremely preterm infants. As a part of this study, we were able to quantify the degree to which staff recorded BP varied from continuously downloaded BP data and investigate the difference between non–invasive BP and invasive BP with the current monitoring systems in our neonatal unit.

This successful pilot study gave us useful information regarding consenting, recruitment, treatment and monitoring which will help in planning and carrying out larger multi centre trials in the future.

4.4 Future work in this area

i. To collect neurodevelopmental outcomes at 2 years corrected age from case records in the first instance and consider testing cognitive function after 5 years corrected age using specific intelligence scale for children if sufficient funding is available.

ii. To examine the correlation between EEG and BP in short epochs.

iii. To carry out a larger confirmatory trial using information gained from this pilot study.

As a result of successful completion of this pilot study and learning from the experience of researchers, parent and clinician feedback, the following paragraphs describe some of the key points that will be considered when planning this larger trial.

4.4.1 Study design

A 3–arm trial comparing BP intervention levels which are higher and lower than the most widely practiced BP intervention level should be considered. BP intervention trials should offer some form of treatment as opposed to placebo–controlled trials which are less likely to be successful in recruiting infants. Parents and clinicians are more willing to let infants participate if there are sufficient safety netting procedures in place to detect low flow states such as cardiac output, NIRS or EEG monitoring. Though this design may not answer the effects of no treatment for low BP, it will encourage parents to let infants participate in the trial.

Using a waiver of consent or a 2–stage consent is something that can be considered which will help infants to be managed using one of the arms from the very start of cardiovascular management. However, experience from this study has shown that parents wished to obtain written consent prior to enrolling their infants.

Further work should examine the effect of BP and its effects on adverse cranial ultrasound outcomes. In addition to this, although expensive, neurodevelopmental outcome testing is a mandatory part of future trials.

4.4.2 Methods

Sample size calculations

The cranial ultrasound outcomes from this pilot study and survival without neurodisability data from previous study (Moore et al. 2012) was used to calculate sample size for future confirmatory trials. The details of this is shown in table 4.1.

Outcome parameters	Proportion obtained from this pilot/ other study	Expected difference in proportions for future trials	Sample size (n) in each group
Grade 2–4, PVL or parenchymal cyst	8/60 (13%)	10%–20%	197
Death or parenchymal brain abnormality	11/60 (18%)	12%–24%	157
Survivors without neurological deficit*	34%	25%–45%	150

Table 4.1: Table showing the incidence of different outcome parameters found in this pilot study and other study which was used to calculate sample size for future confirmatory trials. *(Moore et al. 2012)

Sample size calculations were performed for 80% power at a 5% significance level, with 2-sided tests in a 3-armed study design, without correction for multiple comparisons. Based on the above difference in proportions a sample size of 200 is required for each group, i.e a total of 600 infants.

Blood pressure

Only infants with invasive arterial lines who are likely to survive beyond 24 hours should be considered for participation in the trial. Analysis of BP should include continuously downloaded BP data only. Researchers should expect the difference in BP to occur in the first 48 hours and aim to recruit infants as early as possible in order to study the transitional circulation.

Physiological parameters

Physiological measurements should be carried out on multiple occasions including during extremes of BP in order to capture a wide range of physiological scenarios so that the true relationship between the various physiological parameters can be understood. Continuous

measurement of cerebral blood flow can be considered using near infra red spectroscopy may be considered if resources and sufficiently trained personnel are available.

When multiple centres are involved in large trials, measurement of the various physiological parameters using Doppler ultrasound are best restricted to a few centres in order to reduce inter–observer variability and these parameters can be analysed as a sub–group nested within a study.

Markers of peripheral perfusion such as lactate levels of > 4 mmol/L will increase the sensitivity and specificity for identifying infants with low flow states.

4.4.3 Feasibility of larger confirmatory trial

The successful completion of this pilot study, with a design acceptable to parents and clinicians, has given us the confidence and information in designing and carrying out a larger confirmatory trial.

The rate limiting step for conducting a larger confirmatory trial is the availability of full funding especially when there is an ongoing placebo–controlled BP trial. However, as this study plans to explore BP intervention levels which are higher and lower than the most commonly used BP intervention level practiced in the United Kingdom, this 3–arm BP trial design may be attractive to potential funders.

Another challenging area is achieving complete recruitment of the planned number of infants especially when previous studies were unsuccessful (Batton et al. 2012). As this is not a placebo–controlled trial and BP thresholds being practiced widely are offered along

appropriate safety–netting, parents and clinicians will feel assured and more willing to let infants participate. Approaching as many eligible infants will be the key to successful recruitment in this study.

Though the pilot study was carried out in a single centre, carrying out a 3–arm trial in multiple centres could be potentially challenging especially for clinicians to follow different BP thresholds. Our experience has shown that having regular training sessions with clinicians and including gestation specific written guidelines on when to commence and wean inotropic support at the cot–side has helped with this. In addition, treating all infants with the same medication was helpful and not confusing. Randomisation could be made easier using online tools and with adequate clinician training correct randomisation with minimal protocol violation could be achieved.

Chapter 5

Conclusion

5.1 Conclusion of the study

This thesis describes the relationship between several physiological parameters in the extremely preterm infants in the first three days of postnatal life and is one of the first pilot randomised controlled trial comparing BP intervention levels in extremely preterm newborn infants that completed its planned recruitment. This study is unique in that it compared the three most commonly practiced BP intervention levels (BP intervention levels which were higher and lower than the most commonly used intervention level) and successfully recruited the planned number of infants in an area where previous studies failed due to poor recruitment.

Physiological measurements carried out in this study were related to one another and to electroencephalographic measures of continuity which showed interesting relationships. Cerebral blood flow and cardiac output were noted to increase significantly in the first three days. Cerebral blood flow was directly related to invasive BP whereas cardiac output was inversely related. The relationship between circulatory parameters and commonly used markers of circulatory failure in clinical practice were complex. Electroencephalographic measures of continuity were proven to be significantly related to BP but not through cerebral blood flow. Electroencephalographic continuity was also found to be related to serum carbon dioxide and lactate levels and medications such as morphine.

Inotrope usage was highest in the Active arm and lowest in the Permissive arm, as was invasive day 1 mean BP thus proving the main hypothesis of this study that different BP intervention levels will result in different rates of inotrope usage and levels of achieved BP in extremely premature newborn infants. BP trend for the first week of life was compared between the three arms.

The predefined outcome of haemorrhagic parenchymal infarct occurred only in a very small number of infants making realistic comparisons difficult. The composite cranial ultrasound outcomes in this study was not a predefined outcome and therefore the conclusion we have arrived at using these small number of infants, though demonstrated by other observational studies, should be taken with caution. Larger multi centre randomised controlled trials are required to investigate this association further.

A realistic flow phantom model of the neonatal carotid artery, a first to its kind, was designed. Validity and reliability studies performed on measurement of cerebral blood flow using this flow phantom model demonstrated acceptable validity and reliability.

Research studies on BP management in extremely preterm infants are challenging for a number of reasons, including obtaining consent. Following our experience from this pilot study, we have learned valuable lessons on designing and successfully carrying out similar studies in the future.

Chapter 6

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Chapter 7

Appendix

7.1 Tables

7.1.1 Continuous flow data

No.	Chamber number	Observer initials	Measured diameter (cm)	IWMV (cm/s)	PVE (cm/s)	Actual diameter (cm)	θ / °	High/ Low	Measured flow (ml/min)	Actual flow (ml/min)
1	A1	SP	0.144	20.40	32.80	0.158	50	H	19.934	18.716
2	A1	SK	0.149	20.10	32.50	0.158	50	H	21.029	18.892
3	A1	SP	0.145	9.83	16.80	0.158	48	L	9.739	9.412
4	A1	SK	0.148	9.82	17.00	0.158	48	L	10.136	8.882
5	A2	SP	0.147	17.00	26.90	0.158	46	H	17.311	13.756
6	A2	SK	0.149	16.80	27.10	0.158	46	H	17.576	21.259
7	A2	SP	0.149	8.88	14.20	0.158	46	L	9.290	7.102
8	A2	SK	0.147	8.93	14.20	0.158	44	L	9.093	6.945
9	A3	SP	0.148	13.10	20.50	0.158	38	H	13.522	11.886
10	A3	SK	0.146	14.00	21.40	0.158	38	H	14.063	14.422
11	A3	SP	0.148	7.61	12.30	0.158	38	L	7.855	7.555
12	A3	SK	0.145	8.07	11.80	0.158	38	L	7.996	7.669
13	B1	SP	0.178	29.40	45.20	0.196	48	H	43.896	43.417
14	B1	SK	0.179	31.90	48.10	0.196	46	H	48.166	43.564
15	B1	SP	0.178	11.30	19.20	0.196	50	L	16.872	18.190
16	B1	SK	0.180	10.40	17.00	0.196	48	L	15.879	15.249
17	B2	SP	0.182	29.00	48.70	0.196	46	H	45.267	45.607
18	B2	SK	0.185	27.10	45.00	0.196	42	H	43.707	44.637
19	B2	SP	0.186	14.10	23.50	0.196	44	L	22.987	21.157
20	B2	SK	0.183	15.70	23.30	0.196	38	L	24.777	23.079
21	B3	SP	0.182	29.10	44.40	0.196	38	H	45.423	41.767
22	B3	SK	0.185	28.20	44.20	0.196	38	H	45.481	40.758
23	B3	SP	0.184	11.10	18.70	0.196	38	L	17.709	18.072
24	B3	SK	0.182	11.80	20.30	0.196	40	L	18.419	18.004
25	C1	SP	0.227	18.70	29.80	0.244	44	H	45.408	39.377
26	C1	SK	0.224	20.50	32.10	0.244	48	H	48.472	40.157
27	C1	SP	0.226	7.30	15.10	0.244	50	L	17.570	21.508
28	C1	SK	0.226	10.10	16.50	0.244	46	L	24.310	21.352
29	C2	SP	0.222	16.30	28.90	0.244	44	H	37.856	33.979
30	C2	SK	0.226	16.50	27.50	0.244	40	H	39.714	34.250
31	C2	SP	0.224	8.60	14.70	0.244	44	L	20.335	17.806
32	C2	SK	0.226	6.91	13.40	0.244	40	L	16.632	18.142
33	C3	SP	0.219	19.90	31.10	0.244	38	H	44.976	39.846
34	C3	SK	0.223	18.20	30.70	0.244	36	H	42.650	40.033
35	C3	SP	0.220	8.05	14.90	0.244	36	L	18.360	18.346
36	C3	SK	0.221	9.61	15.10	0.244	34	L	22.118	19.377

Table 7.1: Continuous flow data measured by two independent raters.

7.1.2 Pulsatile flow data

No.	Observer initials	Measured diameter (cm)	IWMV (cm/s)	PVE (cm/s)	Actual diameter (cm)	θ / °	Measured flow (ml/min)	Actual flow (ml/min)	Pump speed	HR
1	SP	0.145	22.6	37.7	0.158	46	22.392	21.551	4	120
2	SK	0.144	22.6	38.4	0.158	50	22.084	21.077	4	120
3	SP	0.144	23.4	38.9	0.158	48	22.866	21.115	4	120
4	SK	0.144	21.5	37.8	0.158	50	21.009	21.866	4	120
5	SP	0.144	26.6	43.3	0.158	50	25.992	23.345	5	150
6	SK	0.148	25.2	43.7	0.158	52	26.011	23.491	5	150
7	SP	0.145	26.2	43.3	0.158	48	25.958	23.457	5	150
8	SK	0.145	27.3	44.0	0.158	50	27.048	23.951	5	150
9	SP	0.146	24.1	38.9	0.158	46	24.208	20.971	4	120
10	SK	0.144	23.4	37.9	0.158	40	22.866	21.146	4	120
11	SP	0.146	26.8	43.1	0.158	44	26.920	23.608	5	150
12	SK	0.146	26.1	43.3	0.158	44	26.217	23.305	5	150
13	SP	0.148	23.1	38.2	0.158	38	23.844	21.515	4	120
14	SK	0.145	21.8	39.2	0.158	42	21.599	20.126	4	120
15	SP	0.144	24.3	39.6	0.158	40	23.745	21.177	5	150
16	SK	0.144	22.8	39.1	0.158	40	22.279	21.948	5	150
17	SP	0.191	12.6	20.8	0.196	44	21.661	19.648	4	120
18	SK	0.187	13.3	21.4	0.196	44	21.917	19.566	4	120
19	SP	0.188	13.8	24.1	0.196	50	22.985	21.78	5	150
20	SK	0.187	15.1	25.3	0.196	50	24.883	21.89	5	150
21	SP	0.190	12.9	21.1	0.196	44	21.945	17.713	4	120
22	SK	0.190	12.3	20.9	0.196	44	20.924	17.928	4	120
23	SP	0.189	11.9	20.4	0.196	40	20.031	18.278	4	120
24	SK	0.189	12.4	21.3	0.196	44	20.873	18.107	4	120
25	SP	0.191	15.0	22.5	0.196	44	25.787	21.004	5	150
26	SK	0.184	14.3	23.7	0.196	42	22.815	21.032	5	150
27	SP	0.186	12.4	24.0	0.196	44	20.216	21.349	5	150
28	SK	0.189	14.9	25.0	0.196	46	25.081	21.28	5	150
29	SP	0.186	12.7	21.0	0.196	38	20.705	18.999	4	120
30	SK	0.185	12.1	19.5	0.196	38	19.515	19.272	4	120
31	SP	0.187	15.0	24.2	0.196	38	24.718	21.701	5	150
32	SK	0.186	12.9	22.0	0.196	38	21.031	21.118	5	150
33	SP	0.225	10.3	17.1	0.244	44	24.572	21.232	4	120
34	SK	0.228	10.3	18.4	0.244	50	25.232	21.746	4	120
35	SP	0.225	11.3	18.7	0.244	50	26.958	22.996	5	150
36	SK	0.227	10.0	18.5	0.244	50	24.282	22.814	5	150
37	SP	0.223	11.6	17.8	0.244	42	27.184	21.52	4	120
38	SK	0.222	10.2	17.8	0.244	44	23.689	20.93	4	120
39	SP	0.222	11.3	19.1	0.244	44	26.244	23.07	5	150
40	SK	0.226	9.07	16.0	0.244	40	21.831	23.398	5	150
41	SP	0.221	10.6	17.9	0.244	40	24.397	21.452	4	120
42	SK	0.225	8.41	14.4	0.244	38	20.063	21.39	4	120
43	SP	0.219	9.20	16.8	0.244	40	20.793	21.534	4	120
44	SK	0.222	8.71	15.8	0.244	40	20.229	20.685	4	120
45	SP	0.223	12.3	19.4	0.244	40	28.824	23.761	5	150
46	SK	0.223	11.2	18.8	0.244	40	26.246	23.628	5	150
47	SP	0.221	12.2	19.3	0.244	38	28.079	23.229	5	150
48	SK	0.223	11.5	18.9	0.244	38	26.949	23.155	5	150

Table 7.2: Pulsatile flow data measured by two independent raters at different pump speeds.

7.1.3 Comparison of invasive and non–invasive blood pressure measurements

No.	GA	B Wt	UAC mean BP	Cuff mean BP arm	Cuff mean BP leg	Average[UAC,Cuff arm]	Average[UAC, Cuff leg]	Diff[UAC-cuffarm]	Diff[UAC-cuffleg]
1	23	670	40	34	53	37	42.3	6	-13
			40	38	34	39	37.3	2	6
			40	38	33	39	37.0	2	7
			40	39	37	39.5	38.7	1	3
			39	47	32	43	39.3	-8	7
			40	46	32	43	39.3	-6	8
2	24	600	32	34	39	33	35.0	-2	-7
			35	45	48	40	42.7	-10	-13
			34	35	41	34.5	36.7	-1	-7
			33	39	48	36	40.0	-6	-15
			34	39	48	36.5	40.3	-5	-14
			34	42	42	38	39.3	-8	-8
3	24	610	27	32	23	29.5	27.3	-5	4
			27	30	25	28.5	27.3	-3	2
			35	38	34	36.5	35.7	-3	1
			30	46	36	38	37.3	-16	-6
			32	41	37	36.5	36.7	-9	-5
			29	47	50	38	42.0	-18	-21
			30	49	21	39.5	33.3	-19	9
			30	46	33	38	36.3	-16	-3
			27	50	27	38.5	34.7	-23	0
			30	43	36	36.5	36.3	-13	-6
			30	45	30	37.5	35.0	-15	0
			30	55	36	42.5	40.3	-25	-6
4	27	980	33	43	35	38	37.0	-10	-2
			30	36	38	33	34.7	-6	-8
			32	36	37	34	35.0	-4	-5
			30	40	43	35	37.7	-10	-13
			31	40	44	35.5	38.3	-9	-13
			32	37	53	34.5	40.7	-5	-21
5	28	1010	38	40	39	39	39.0	-2	-1
			44	44	45	44	44.3	0	-1
			38	49	43	43.5	43.3	-11	-5
			34	41	31	37.5	35.3	-7	3
			32	46	46	39	41.3	-14	-14
			35	53	38	44	42.0	-18	-3
6	28	770	30	48	44	39	40.7	-18	-14
			30	36	35	33	33.7	-6	-5
			32	44	43	38	39.7	-12	-11
			32	40	44	36	38.7	-8	-12
			33	42	49	37.5	41.3	-9	-16
			33	40	47	36.5	40.0	-7	-14
7	38	3560	38	50	48	44	45.3	-12	-10
			36	46	48	41	43.3	-10	-12
			38	50	50	44	46.0	-12	-12
			37	52	52	44.5	47.0	-15	-15
			38	55	50	46.5	47.7	-17	-12
			33	47	45	40	41.7	-14	-12
8	24	665	39	49	47	44	45.0	-10	-8
			41	49	59	45	49.7	-8	-18
			40	41	59	40.5	46.7	-1	-19
			41	42	43	41.5	42.0	-1	-2
			41	44	47	42.5	44.0	-3	-6
9	25	780	30		38		34.0		-8
			31		33		32.0		-2
			38		40		39.0		-2
			36		44		40.0		-8
			37		35		36.0		2
			42		27		34.5		15
			40	41		40.5		-1	
10	25	740	34	49		41.5		-15	
			32	35		33.5		-3	
			32	38		35		-6	
			29	37		33		-8	
			33	39		36		-6	
			30	43		36.5		-13	
11	27	640	21	47		34		-26	
			25	58		41.5		-33	
			26	59		42.5		-33	
			24	58		41		-34	
			25	56		40.5		-31	
			27	51		39		-24	
12	24	640	25	38		31.5		-13	
			20	36		28		-16	
			21	36		28.5		-15	
			28	37		32.5		-9	
			26	47		36.5		-21	
			23	43		33		-20	

Table 7.3: Table illustrating the mean blood pressure measurements from randomly selected infants admitted to the neonatal uint. All mean BP figures in mmHg

7.1.4 Table showing normality of data for variables measured in the study

Clinical parameter	Normality of data
Gestation	Normal
Birth weight	Normal
Cord gas	Normal
Apgar score	Normal
Temperature on admission	Normal
Continuously downloaded mean BP	Normal
Staff recorded mean BP	Normal
Duration of inotropes	Non–normal
Maximum dose of dopamine	Normal
Duration of UAC	Normal
Days at each level of care	Normal
Serum creatinine	Non–normal
Serum potassium	Normal

Table 7.4: Normality of data for clinical parameters examined using histogram, Q-Q plot and Kolmogorov–Smirnov test.

Physiological parameters	Normality of data	
	Day 1	Day 3
pH	Non–normal	Normal
$PaCO_2$	Non–normal	Non–normal
Serum lactate	Non–normal	Non–normal
RCCA blood flow volume	Non–normal	Non–normal
SMA flow velocity	Normal	Normal
Cardiac output	Normal	Normal
Maximum EEG amplitude	Normal	Normal
Minimum EEG amplitude	Normal	Normal
% minimum amplitude < 5 μV	Non–normal	Normal
Mean interburst interval	Normal	Non–normal

Table 7.5: Normality of data for physiological parameters examined using histogram, Q-Q plot and Kolmogorov–Smirnov test.

7.2 Study protocol

A pilot randomised trial comparing intervention levels for the support of blood pressure in extremely premature newborn babies

Aims

The aim of this project is to compare different levels of blood pressure at which staff caring for extremely premature newborn babies should intervene to start treatment. This is a preliminary study which will determine whether active, moderate or permissive blood pressure support regimens results in different achieved blood pressure levels and different rates of inotrope usage. The data collected in this study will determine whether a larger multicentre study would be feasible and justified. Secondary outcome measures will be collected to inform power calculations for important clinical outcomes in a larger study.

Three approaches to intervention will be compared in babies 23-28 weeks gestation:

1. **Active:** Support blood pressure if it falls below 30mmHg
2. **Moderate:** Support blood pressure if it falls below the baby's gestation in mmHg
3. **Permissive:** Do not intervene unless hypotension is accompanied by evidence of impaired perfusion

(i) Background

Blood pressure is routinely monitored in all neonatal units, but the criteria for supporting low blood pressure vary widely. A number of trials have examined the drugs used to support blood pressure, but there have been no randomised studies comparing blood pressure intervention levels. International review groups have identified this as a priority for research[1].

Of the 2528 neonatal deaths in England and Wales in 2005, many are related to problems which could be amenable to support of the circulation[2]. These include disorders related to short gestation and low birth weight (1055 deaths), respiratory and cardiovascular disorders originating in the perinatal period (93 deaths), bacterial sepsis of the newborn (102 deaths), intraventricular haemorrhage (over 100 deaths) and necrotising enterocolitis (68 deaths).

There is a wide range of opinion in neonatal care as to whether blood pressure is a parameter of marked importance, or one which is peripheral to the main aims of therapy. Historically, the focus has moved from non-specific circulatory support (e.g. intravascular volume), to support of blood pressure, to maximisation of cardiac output and more recently, the support of organ-related blood flow parameters such as superior vena cava (SVC) flow. Early studies of blood pressure and brain injury in premature babies suggested a link between low blood pressure and parenchymal brain injury[3]. In the next phase of research it was assumed that low blood pressure was a marker of low cardiac output, and that support for hypotension should aim to increase cardiac output.

However, it has become clear that blood pressure, cardiac output and organ specific blood flow may all vary independently of each other. Indeed, randomised studies show that inotropic agents such as dopamine may increase blood pressure without increasing cardiac output, whereas dobutamine may elevate cardiac output without increasing blood pressure[4]. Physiological studies examining the relationship between blood pressure and brain blood flow in sick premature infants have yielded conflicting results which have not helped to resolve this dilemma. Although some studies show that brain blood flow or oxygenation are related to arterial blood pressure[5,6,7] others have documented no such relationship[8]. Low cerebral blood flow does seem to be a risk factor for severe intracranial haemorrhage[9]. There is even less data on the effect of blood pressure and cardiac output on the perfusion of other vital organ such as the heart, liver, gut and kidneys in premature neonates.

At present there is no continuous bedside measure of organ perfusion which has achieved widespread use in premature infants and clinical measures of perfusion such as capillary refill time are no better than blood pressure in detecting low upper body blood flow[10]. There are difficulties with the reproducibility of SVC flow and cardiac output[11] which mean that these measures can often only be measured by a few individuals on any neonatal unit. Even when such technical issues are resolved, blood pressure may still prove to be an important parameter. Certain organ functions, such as the production of urine through glomerular filtration in the kidneys, may be pressure-dependant. Our own data shows lower values for creatinine in neonates where blood pressure was maintained above 30mmHg compared with published data from other units[12]. Similarly, even if global brain blood flow is preserved, there may be 'watershed' areas in which brain blood flow will be inadequate.

There are also practical reasons explaining why an adequate blood pressure is difficult to define for extremely premature infants. With term infants a healthy population can be used to define normal ranges of blood pressure, with values outside of this range deemed abnormal and an indication for intervention. For extremely preterm infants it is hard to define a 'normal healthy' population as most are at risk of developing conditions related to poor organ perfusion.

(ii) Intervention levels currently in use

In view of the considerable uncertainty about the levels at which blood pressure support should be started, a pragmatic guideline was recommended by a BAPM (British Association of Perinatal Medicine) working group some years ago, which has gained widespread acceptance. This group suggested that blood pressure should be maintained above the baby's gestational age in mmHg (i.e. blood pressure should be maintained above 25mmHg for a baby of 25 weeks gestation). In a recent survey, this was found to be the most common regimen in clinical use in the UK[13].

A reasonably rational approach of intervening when blood pressure falls below certain centiles in extremely preterm babies is also used, but is not widespread, partly due to practical difficulties in applying criteria which vary with size, maturity and postnatal age of the baby.

In 1993, our own unit introduced a more active level of intervention (30mmHg in all preterm babies) based on Miall-Allen's study showing more parenchymal brain lesions in infants who experienced blood pressures below this level[3]. This was formally integrated into the written agreed unit guidelines for all patients from 2001, with recommendations on staged treatment for hypotension, including use of echocardiographic assessment. An integrated cardiovascular support guideline encompassing hypotension and low cardiac output was implemented in 2007.

With a paucity of evidence to support particular intervention levels, and conflicting physiological evidence on the relationship between blood pressure and cerebral blood flow, others have suggested that blood pressure should be ignored in neonatal intensive care, so long as simple clinical parameters do not demonstrate poor perfusion. This approach has been termed 'permissive hypotension' and has been actively championed in a cohort study published by Dempsey et al[14] who used this approach in a tertiary neonatal unit in Montreal, Canada. Despite the limitations of such non-comparative cohort studies, some UK neonatal units are now adopting this approach.

(iii) Comparison of permissive hypotension with active support for hypotension

We are currently evaluating the 'permissive hypotension' approach against own, more active 30mmHg approach in two ways. We are comparing clinical outcomes in extremely preterm babies with another nearby tertiary unit which uses permissive hypotension, trying to relate these outcomes to achieved blood pressures and inotrope use. This work is being supported by an NIHR grant awarded to the Homerton Hospital which was applied for jointly.

We have also compared our own unit's outcomes with those published by Dempsey et al, using identical patient selection criteria. Their series was studied during 2000-2003; we have compared outcomes during a comparable five-year period after we introduced our integrated written cardiovascular support guidelines dealing with the treatment of hypotension and poor cardiac output (2001-2005). The results of this comparison are shown in Table 1 overleaf.

	All patients in cohort				Patients in cohort receiving inotropes		
	Permissive hypotension	Active support <30mmHg	Permissive vs active p-value		Permissive hypotension	Active support <30mmHg	Permissive vs active p-value
Years	2000-3	2001-5			2000-3	2001-5	
n	118	141			18 (15%)	97 (69%)	**<0.001**
Gestation (SD)	26.1	25.9 (1.9)	-		25.2 (1.6)	25.2 (1.6)***	-
Birthweight (SD)	780	760 (146)	-		728 (149)	725 (142)***	-
M:F	-	70:71	-		-	51:46	-
Antenatal Steroid	73%	86%	**0.01**		65%	84%	0.11
Vaginal delivery	34%	43%	0.12		32%	46%	0.31
PDA	63%	41%	**0.001**		37%	53%***	0.29
Outcomes							
Confirmed NEC	11 (9%)	17 (12%)	0.48		2 (11%)	13 (13%)	1.00
Surgical NEC	3 (3%)	6 (4%)	0.52		1 (6%)	6 (6%)	1.00
Isolated GI perforation	3 (3%)	8 (6%)	0.35		1 (6%)	8 (8%)	1.00
Cystic PVL	1 (1%)	5 (4%)	0.22		0	3 (3%)	1.00
IVH 3-4	13 (11%)	22 (16%)	0.28		5 (28%)	20 (21%)**	0.54
Mortality	33 (28%)	30 (21%)	0.21		13 (72%)	24 (25%)	**<0.001**
Survival without above adverse outcomes	77 (65%)	81 (57%)	0.20		4 (22%)	47 (49%)	**0.04**
			Chi-squared or Fisher's exact test		Inotrope group vs those not receiving inotropes (*=p<0.05; **=p<0.01; ***=p<0.001)		Chi-squared or Fisher's exact test

Table 1: Comparison between the Montreal extremely low birth weight (ELBW) cohort published by Dempsey et al (2009) and outcomes for babies admitted to our own neonatal unit. Dempsey studied patients admitted during a 4-year period 2000-2003. Patients from our own unit were admitted during a similar 5-year period immediately after introducing a formal printed guideline for management of hypotension (2001-2005). Both series include only babies admitted on the first day of life and exclude lethal congenital malformations. This data includes the four patients in the Montreal series who died in the first four hours of age without blood pressure being measured, and all deaths between admission and discharge home in our own cohort.

The two cohorts had similar birthweight and gestation, with significantly higher antenatal steroid administration in east London and significantly higher rate of diagnosis of patent ductus arteriosus in Montreal.

For all the patients, the marginally lower mortality in London was clearly not statistically significant from that in Montreal (21% vs 28%, p=0.21). The slightly higher rate of favourable composite outcome (survival without necrotising enterocolitis, gastrointestinal perforation, grade 3-4 periventricular haemorrhage or cystic PVL) in Dempsey's series was also not significant (65% vs 57%, p=0.20). In terms of non-significant trends, one could argue that gastrointestinal complications were slightly higher in the active treatment cohort. One should be very careful not to over-interpret such non-significant trends, especially given the small numbers and the likely major differences in patient factors between Montreal and east London.

Two findings stand out as very different between the two cohorts. Firstly, a much higher proportion of patients received inotropes with the active support regimen. Secondly, among the babies who received inotropes, rates of mortality and complications were much better among the patients in the active treatment cohort. Those favouring this regimen could argue that if inotropes are to be given, they should be started in response to minor degrees of hypotension. Those favouring permissive hypotension could argue that many of the babies actively treated would have done well even if they had not received inotropes.

The study of Dempsey et al used a composite outcome of mortality and various short-term outcomes. An implication of the short-term outcomes is that survival with these outcomes may be undesirable because of association with poor neurological outcome. We had developmental outcome data on 76% of the surviving infants with these short-term outcomes. Of these infants, 31% had disability at follow-up, 12% had some impairments without disability and 58% had no disability or impairment. We therefore question whether these short-term outcomes can be used as surrogate markers of important long-term clinical outcomes.

Because non-randomised comparisons between units are unlikely to determine the superiority of any particular approach, randomised trials now provides the best way of determining whether a more active or a more permissive blood pressure support regimen best reduces complications in premature infants. Maintaining blood pressure at a higher level could improve or stabilise blood flow, reducing the chance of organ damage. However, this could come at the cost of longer, more interventional treatment, hard to justify unless clinical outcomes are improved. Excessively active treatment could compromise blood flow if inotropic drugs cause

vasoconstriction. If a particular level of blood pressure intervention was clearly clinically superior, this easily measured parameter could be rapidly translated into practice in all neonatal units.

We are convinced that the best way to answer this question will ultimately be to conduct a large, multicentre randomised clinical trial. We have previously sought funding for a trial comparing active circulatory support with the most common current UK regimen, but have been unsuccessful. To show the likely small differences in mortality or disability would require very large numbers. To justify the funding for a large trial and to recruit centres, a pilot study is essential to demonstrate that the interventions will produce a clear separation in treatments, achieved blood pressures or physiological outcomes.

This pilot study is therefore being proposed to provide the answers to several important issues which need to be clarified before conducting a large-scale multicentre randomised trial which is adequately powered to answer the question of clinical outcome. These issues are:

1. Do different blood pressure intervention thresholds affect inotrope usage?
2. Do different thresholds affect achieved blood pressure levels?
3. Can we define the incidence of complications and outcome measures in recruited patients to inform meaningful power calculations for larger clinical trials?
4. Is it feasible to carry out a trial involving three different intervention thresholds, or would staff find this too confusing? If it is feasible to include more than two levels in a single study, this could dramatically reduce the number of trials required to answer the question of whether the UK's current blood pressure intervention level in extremely preterm babies is correct, too low or too high.
5. If there are differences in inotrope usage or achieved blood pressure, are these associated with differences in organ-specific perfusion, complications or function which could give indications of the potential benefits of each approach?

(iv) Main Hypothesis

The main hypothesis is that different approaches to blood pressure intervention will result in different usage rates of inotropic agents and levels of achieved blood pressure, in extremely premature newborn infants (less than or equal to 28 weeks gestation). These findings, together with the rates of complications found in the whole study, will be used to design a larger multicentre study.

(v) Methods

The study hypothesis will be tested in a single-centre open randomised trial with mainly clinical outcome measures. To determine the effects on blood flow as well as blood pressure, babies will also have detailed physiological studies whenever possible.

Patient eligibility

Patients will extremely preterm infants who will be eligible to take part in the study if they are:

- Born at less than or equal to 28 weeks gestation
- Admitted to our neonatal unit
- No known major congenital malformation
- Recruited and randomised in the first 12 hours of life

There will be no restriction on the method used to measure blood pressure, although invasive monitoring will be encouraged as the gold-standard. Restricting the study to invasive monitoring would cause unacceptable drop-out when there are delays in establishing arterial access, or when arterial catheters have to be removed. Guidance will be given on the interpretation of invasive and non-invasive blood pressure measurement, to minimise the use of spurious measurements. To maximise recruitment, patients transferred ex-utero on inotropic therapy will be included, with weaning of support if their blood pressure is above the randomised level. There will be a planned sub-group analysis for this group.

Consent

Information will be given to the parents when extremely premature delivery is anticipated and signed assent obtained to prepare them for the formal consent process. Formal written informed consent will be obtained following delivery of the baby. For unexpected or precipitate deliveries, parents will be approached, given information and consented after delivery.

Randomisation

Randomisation will be online using gestation stratification in permuted blocks. Standard treatment will be instituted according to the unit's established guidelines for cardiovascular support when the patient reaches their allocated intervention level of mean arterial blood pressure (MABP), which will be randomly assigned to one the three following options:

1. **Active:** If the baby's MABP falls below 30mmHg for more than 15 minutes
2. **Moderate:** If MABP falls below the baby's gestational age in mmHg (commonest UK criteria) for more than 15 minutes
3. **Permissive** hypotension - blood pressure will not be supported unless there is clinical evidence of impaired tissue perfusion, or unless MABP falls below 19mmHg for more than 15 minutes.

Data collection and study measurements

Clinical outcome measures, including blood pressure levels and treatment, will be collected onto a standard proforma during the infant's stay. Although invasive measurements would be the gold standard for measurement of blood pressure, it is not unusual for arterial line blockage or removal to occur and therefore we will have to resort to non invasive blood pressure measurements in these babies. Our past internal audit of the Phillips monitor used in our unit showed that they tend to under-read blood pressure (rather than over-estimate it, as is the case with some other monitors). In the instances where non invasive blood pressure has been used, these will be specified in the analysis of the results and data on pure invasive measurement will be provided.

All babies will have cardiac output and right common carotid artery blood flow measured on day 1 of age as a safety net to ensure that babies with impaired tissue perfusion are identified. Clinicians looking after the baby will be informed if the cardiac output is less than 150ml/kg/min or if the right common carotid artery blood flow volume is less than 2 SD below the mean. To avoid a clinical information bias between the groups, this safety net will be provided for all patients in the study.

As many patients as possible will also have physiological variables measured using echocardiography and an amplitude-integrated EEG performed at 3 days of age. Cranial ultrasound scans performed at one week of age and 34-36 weeks gestation.

Weaning of inotropic support

Blood pressure support will be escalated until the BP stabilises above the intervention level. Once the blood pressure is above this level, weaning will take place as follows:

1. If BP > 10 mmHg above intervention level for 15 minutes, wean inotropic support immediately by at least 20% and continue to wean 1-2 hourly whilst BP stays at this level.
2. If > 5 mmHg above the intervention level, begin to wean after 6 hours of stability at this level and continue to wean 2-4 hourly whilst BP stays at this level.
3. If 1-5 mmHg above intervention level, begin to wean after 12 hours of stability and continue to wean 2-4 hourly whilst BP stays at this level.

Primary outcome measures

1. Mean arterial blood pressure during the first week of life (collected hourly for the first 12 hours and 4-hrly thereafter)
2. Use of inotropes and duration of their use

Secondary outcome measures

1. Death or parenchymal brain abnormality on cerebral ultrasound before discharge home
3. Death before discharge home from hospital
4. Periventricular leucomalacia (on cranial ultrasound at or before 36 weeks corrected gestational age)
5. Parenchymal periventricular haemorrhage (on cranial ultrasound on Day 1 and by one week)
6. Other periventricular haemorrhage
7. Acquired gastrointestinal pathology (necrotising enterocolitis, perforation or GI surgery)
8. Treatment for patent ductus arteriosus (drugs or ligation)
9. Maximum serum creatinine in the first 2 weeks of life
10. Maximum serum potassium level
11. Duration of respiratory support
12. Chronic lung disease (defined as oxygen dependency at 36 weeks post-conceptual age)
13. Use of postnatal steroids including hydrocortisone
14. Duration of neonatal care at each BAPM care level (this is the basis for charging for care and a good marker of health service costs)
15. Neurodevelopmental status at routine developmental follow-up

Patients with physiological studies

To determine whether observed effects are primarily related to blood pressure or blood flow differences, as many patients as possible will have more detailed physiological studies performed in the first week of life using echocardiography. The measures for this subset will be:

1. Common carotid artery blood flow
2. Left ventricular output and assessment of ductus arteriosus shunt
3. Superior mesenteric artery blood flow velocity
4. Amplitude-integrated EEG
5. Downloaded continuous blood pressure data from monitors

A full set of measurements will be performed at 3 days (48-96 hours of age), when the greatest differences in parameters might be expected to be present. Additional cardiovascular measurements will be performed in selected patients at other pertinent times, such as when extremes of blood pressure or treatment are encountered. The measurements of common carotid artery blood flow will use a technique which we have validated[15].

Statistical Analysis

All outcomes will be analysed with consideration of study randomisation and gestational age. Analysis of variance will be used for parametric measures (blood pressure, duration of inotrope usage) with gestation as a covariant. For categorical measures (survival, intracranial pathology) chi-squared test will be used for primary analysis, with logistic regression to determine the effects of randomisation, gestation, birth weight and other perinatal factors. There will be a planned sub-group analysis with patients already on inotropes at randomisation excluded.

Study power

As a pilot study, formal statistical advice was that power calculations were not necessary. However, we wanted to give this pilot study a good chance of showing differences in some of the major outcomes. We therefore performed sample size calculations using data available from our previous studies and comparisons with other published data:

For the primary outcome measures of the proportion of babies requiring inotropes, we used data from the comparison of outcomes between our unit and Dempsey et al's published data (see protocol background). Comparison of our data with that of Dempsey et al showed inotrope usage of 15% and 68% respectively. As differences are likely to be slightly smaller in a randomised trial, we have calculated sample size on the basis of a 20%-60% difference in proportions, analysed using chi-squared test. In a two-treatment study, 20 patients would be required in each group to demonstrate a difference between 20% and 60%, with 80% power and a 5% significance level.

We have also carried out power calculations for a 3-level, one-way ANOVA analysis of the parametric secondary outcome of common carotid artery flow. Our previous study on common carotid artery flow showed a mean value of 20ml/kg/min in stable preterm infants, with a standard deviation of 4.9ml/kg/min. It would require 20 patients to demonstrate a difference of 5ml/kg/min, with 80% power and a 5% significance level. Calculations were performed using Minitab v16.1.0, Minitab Inc 2010.

We are therefore aiming to recruit 20 patients to each arm of the study (a total of 60 patients).

Serious Adverse Events

Serious Adverse Events (SAE's) will be defined as death in the first 28 days of life or bilateral parenchymal periventricular haemorrhage. Suspected Unexpected Serious Adverse Reactions (SUSAR's), SAE's and primary and secondary clinical outcomes will be monitored by the research team and notified to the DMC.

Ethics and Host institution

Barts and the London School for Medicine and Dentistry will provide research governance and indemnity. The trial will be registered with the MCRN and the UKCTG Clinical Trials database.

(vi) Relationship to other work by this group

For the applicants, this work relates closely to our work on carotid blood flow and mesenteric perfusion in premature infants. In addition, Dr Kempley has recent experience of multicentre randomised trials as a co-applicant and core investigator for the national ADEPT trial.

Dr Sinha has developed the carotid blood flow measurements; the study of regional circulations remains an area of research in the unit. Dr Kempley and Dr Sinha are co-applicants on a current NIHR award which is supporting staff at The Homerton Hospital (Dr Aladangady) in a comparison of outcomes between the two units. Dr Shah has an established track record in aEEG and other neonatal neurology research.

(vii) Relationship to work by other groups

There are currently no published randomised trials evidence comparing different blood pressure thresholds for cardiovascular support, despite the recommendation that this should be an area for research[1]. The systematic Cochrane reviews in this area do not examine blood pressure intervention levels. The review of inotrope use in low systemic flow found only one study with 42 patients[16]; the review comparing dopamine with dobutamine for hypotension in preterm infants, included less than 200 infants and did not show differences in mortality or long term outcome[4].

A research program led by Dr Eugene Dempsey in Cork, Ireland (the HIP trial, http://www.hip-trial.com) has recently received EU FP7 funding (FP7/2007-2013) to compare permissive and moderate intervention levels. Although this trial aims to recruit 800 patients from centres in Canada and Europe, we are concerned that it will only compare the permissive and moderate approaches. At the end of this trial, it will still not be certain as to whether a more active approach than commonly practiced in the UK would provide better outcomes.

(viii) Difficulties anticipated

The greatest difficulty in this trial will be to obtain informed consent at a critical time of life for the baby and a worrying time for parents. We are asking the opinion of parents as to their opinion of the research study and the best approach to informed consent and randomisation. However, approached sensitively, this has been successfully accomplished in other trials we have performed. The study will use treatments in standard clinical use, with no new drugs.

(ix) Further work expected

Permission will be obtained for collection of information on neuro-developmental outcome by questionnaire from clinicians or parents at 2 years of age. The trial may show effects on secondary outcomes which seem important but fall short of statistical significance. This data would provide justification and preliminary data modelling for a much larger multicentre trials. If there is not a clear separation of treatments and achieved blood pressure, indicating contamination effects with a

confusion of staff between allocations, this would suggest that a different approach, such as cluster randomisation of centres or time time periods may be required.

(x) Justification for salaried posts and expenses

The Clinical Fellow is currently funded by NHS funding and has been granted OOPE for this placement. This has been justified to balance Nurse Practitioner clinical time which is currently biased to provide daytime cover, to allow these staff to contribute to the middle grade rota. Grant funding for continuation of the Fellow post has been applied for. A least 50% of their time will be spent in research, with the remaining clinical time mainly contributing to out-of-hours cover. The 50% research time will provide adequate opportunity for recruitment, clinical measurements, data connection and analysis.

(xi) Clear outcomes to drive larger studies

The following outcomes will provide 'stop-go' rules for future studies:

- If recruitment rates are less than 30%, this will show that an individually randomised trial cannot provide a representative sample for the interventions. In this case, the only way to study these interventions would be through a cluster randomised trial.
- If there are no significant differences in achieved blood pressure or inotrope usage, suggesting treatment contamination between randomised groups, this would again suggest that a cluster randomised approach should be used.
- If there are recruitment rates of 50% and differences in achieved blood pressure levels, this would support an individually randomised study as the most valid study design.
- The secondary outcome measures from the whole study group will be used for power calculations of larger studies, as complication rates will be typical of recruited patients in a randomised trial.
- When recruiting other centres in future studies, physiological data will be vital driver in showing mechanisms of clinical effects and the basic safety of different intervention levels. If two of the intervention levels show identical treatment and physiological effects, this would drive the omission of one of these approaches from next phase studies.

Appendix: References

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7.3 Parent information sheet

Barts Health NHS
NHS Trust

Version 2 24/10/2012

Parent Information Sheet
A pilot randomised trial comparing intervention levels for the support of blood pressure in extremely premature newborn babies

We are inviting you to allow your baby to take part in a research study. Before you decide, it is important to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Please ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study and why have I been chosen?

This study will examine when we should support low blood pressure in premature newborn babies. We will be comparing different levels of blood pressure at which staff will intervene with standard supportive treatment. You have been selected because your baby is premature, or because they may be born prematurely.

We already know that blood pressure is much lower in premature babies than in older children and adults. Some of the problems faced by premature babies may be related to low blood pressure. Once we decide to support blood pressure, there are effective treatments to bring the blood pressure up to higher levels. This hospital has clear guidelines on how to treat blood pressure in premature babies, in place for many years, but we wish to compare our existing practice with alternative approaches.

Currently, there is no agreement on how low we should allow blood pressure in preterm babies to fall, before we step in to support it. This is an initial study to find out how different approaches affect the baby's blood pressure and their treatment.

What will happen to my baby if I agree for them to take part?

If you agree to your baby to take part, they will be allocated to a particular blood pressure intervention level by a process called **randomisation**. This is used in research studies when we don't know which way of treating patients is best. To find out, we need to make comparisons between the different treatments. We put people into groups and give each group a different treatment; the results are compared to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly).

For this study, equal numbers of babies will be randomly allocated to one of the following blood pressure groups:
1. Active: Staff will support blood pressure if it falls below 30mmHg (our current policy in this hospital)
2. Moderate: Staff will support blood pressure if it falls below the baby's gestation in mmHg (for example, 25mmHg for a baby of 25 weeks)
3. Permissive: Staff will only intervene to support low blood pressure if there are signs of poor blood flow or if blood pressure falls below 19mmHg

All of these levels are being practised in different neonatal units across the UK. At present, there is no evidence that any one level is better than the other.

What happens after my baby is allocated to their blood pressure level?

Once a baby needs support for their blood pressure, they will all receive the same treatments, similar to our existing guidelines. This will involve giving fluids and standard drugs called inotropes. (The most commonly used drug is called dopamine).

On the 1st and the 3rd day after birth, your baby will have a detailed ultrasound scan to look at blood flow from the heart to the brain and bowel. They will also have a test examining the electrical activity of the brain (an EEG). In some babies an additional ultrasound scan will be carried out if they have a particularly low or high blood pressure. The ultrasound scans are similar to the scan you may have had during your pregnancy, and the scans babies routinely have on the Neonatal Unit.

All babies will be carefully monitored as part of their standard care and we will collect detailed information on their blood pressure, treatment and progress from your baby's records. The study is being carried out by the qualified medical and nursing staff at the Royal London Hospital and will recruit a total of 60 babies.

The research interventions will be completed well before your baby is due to go home from hospital. At this time we will ask your opinions on taking part in this study. After your baby goes home, we will collect information on their progress at 1 and 2 years of age. We will send you a questionnaire on their progress, and we will collect information from the doctor seeing them in their routine outpatient follow-up clinics.

What are the possible benefits of taking part?

We cannot promise the study will help your baby personally, but the information we get might help improve the treatment of premature babies. Your baby will have the benefit of additional monitoring from the research team.

What are the possible disadvantages and risks of taking part?

The only additional tests which your baby will need for the research will be the scans and EEG. The ultrasound scans involve placing a small amount of jelly on the surface of the baby's skin on the chest and on the neck, followed by gently resting a probe on this jelly. The test of the brain's electrical activity (EEG) involves placing 4 electrodes on the surface of the scalp in the baby's hair, again using some jelly. The scans and monitoring will allow us to make sure that low blood pressure is not affecting blood flow in your baby.

Will any blood tests or samples be taken?

No. The research will not result in your baby having any additional blood tests or investigations, apart from the scans. However, all premature babies do have regular blood tests and scans as part of their standard care.

What do I have to do?

You will have to let us know whether or not you will let your baby take part in the study and sign the consent form before your baby is 12 hours old. This is needed so that staff can be definite about how to support your baby's blood pressure. Our medical and nursing staff will monitor your baby's blood pressure and start treatments if they are needed. We will ask you to fill in the questionnaires before your baby goes home from hospital, and at 1 and 2 years of age

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NHS Trust

Version 2 24/10/2012

Other general information about the research

Does my baby have to take part?

No. It is up to you to decide whether or not to allow your baby to take part. If you do, you will be given this information sheet to keep and you will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your baby receives. We would normally analyse any information collected up to the point of withdrawal.

What are the alternatives for diagnosis or treatment?

If your baby does not take part in the study, they will receive the standard support for blood pressure for this hospital, the same as the 'Active' level detailed above.

What will happen with the results of the study?

We will compare short and long term complications as well as organ blood flow between the groups. We do not expect this study to definitely tell us which intervention level should be used, but this information will be used to design a larger trial in more hospitals which may be able to answer this question. The results of this study will be published in medical journals. If you would like to see the results, we can also provide these to you when they are available. We will not identify you or your baby in any report/publication

Will my baby taking part in this study be kept confidential?

All information collected about you and your baby during the course of the research will be kept strictly confidential. If you consent to take part in the research the people conducting the study will abide by the Data Protection Act 1998, and the rights you have under this Act.

The research staff employed by this hospital will collect information as follows:

- Paper forms will have details of your baby's name, hospital number and NHS number. These will be kept in locked cabinets and offices in the hospital.
- Information will be kept in password-protected files on the secure hospital computer system, with details of your baby's name and hospital number, information about their progress, scan results and blood pressure profiles.
- For computerised analysis of results, your baby will be identified by a study code and all identifiable data such as names and NHS number will be removed. This analysis of 'anonymous' data will be performed on secure Medical School computers.
- No identifiable data will be passed on to third parties. If any data is required for future studies, only fully anonymised data without study codes or names will be made available to anybody outside this hospital and medical school.

All information will be stored securely. Dr Kempley, Consultant Neonatologist and Chief Investigator for this study, will be responsible for ensuring the security of the data. The data will only be accessed by authorised persons such as researchers, sponsors, regulatory authorities & R&D audit (for monitoring of the quality of the research). You will have the right to check the accuracy of data held about your baby and correct any errors.

Involvement of the General Practitioner/Family doctor (GP).

We do not need to contact your GP during the baby's stay in the hospital, but your baby's discharge summary will tell your GP that your baby was in the study.

Version 2 24/10/2012

What happens if there is a problem?

We would not expect your baby to suffer any harm or injury because of your participation in this research. If you or your baby are harmed by taking part in this study, there is no special compensation arrangement. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or if you have a complaint. Please telephone 020 359 42040 or email PALS@bartsandthelondon.nhs.uk, you can also visit PALS by asking at hospital reception.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want your baby to continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your baby's best interests to withdraw them from the study. They will explain the reasons and arrange for your baby's care to continue. If the study is stopped for any other reason, you will be told why and your baby's continuing care will be arranged.

What happens when the research study stops?

Following the study intervention, your baby will receive the normal care given to premature babies within our service.

What if there is a problem and Contact Details for further information:

If you have any concerns about the research during or after the study, please feel free to discuss them with the medical and nursing staff on the Neonatal Unit. You can also ask to speak with the Researchers, who are:

Dr Sujith Pereira }
Dr Steve Kempley }
Dr Ajay Sinha }
Dr Divyen Shah }

Neonatal Unit, Ward 8D,
Royal London Hospital
Whitechapel, London, E1 1BB
Tel: 020 3594 0541 or 020 3594 0524 (out-of-hours)

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you wish to make a formal complaint, please contact: Quality Development, Barts Health NHS Trust, Healthcare Governance Directorate, 3rd floor, Prescot Street, tel 020 7480 4857 .

Who is organising and funding the research and where was it reviewed?

This research study has been developed by the doctors of the Neonatal Unit of Barts Health NHS Trust. It does not have any external funders or sponsors. The study has been reviewed by the London Surrey Research Ethics Committee.

7.4 Consent form

Queen Mary University of London

Barts and The London NHS Trust

CONSENT FORM (Version 3_24.10.2012)

Title of project: A pilot randomised trial comparing intervention levels for the support of blood pressure in extremely premature newborn babies

Investigators: Dr Steve Kempley, Dr Sujith Pereira, Dr Ajay Sinha, Dr Divyen Shah

Study Number: Patient Identification Number for this trial:

Antenatal Assent

I confirm that I have read and understand the information sheet for this study. I have been able to consider the information, ask questions and have had these answered satisfactorily. I provisionally agree to my baby taking part in the study after they are born. I understand that no interventions will be started until I sign to confirm my agreement after my baby is born.		
Name of parent	Signature	Date
Witnessed	Signature	Date

Postnatal Consent

Please initial box to indicate agreement

1.	I confirm that I have read and understand the information sheet dated ..... (version .....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2.	I understand that my participation is voluntary and that I am free to withdraw my baby at any time, without giving any reason, without my medical care or legal rights being affected.	
3.	I understand that relevant sections of any of my baby's medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities or from the Barts and the London/ Queen Mary University of London, where it is relevant to my taking part in this research. I give permission for these individuals to have access to the records.	
4.	I agree to my GP being informed of my baby's participation in the study.	
5.	I agree for my baby to take part in the above study.	
6.	I agree to anonymised data being made available for the planning of future studies.	

Name of Parent	Date	Signature
Name of Person taking consent (if different from Investigator)	Date	Signature
Investigator	Date	Signature

1 copy for Patient, 1 for Investigator and original to be kept in medical notes

Page 1 of 1

7.5 Neonatal unit guideline for management of hypotension

Neonatal Hypotension and Shock

Review Date	February 2011
Approved By:	Neonatal Consultants Neonatal Matron
Original Distribution	All Paediatric and Neonatal Wards
Related Policies	Neonatal Formulary
Written By:	Dr Steve Kempley & Dr Ajay Sinha Consultant Neonatologists February 2008
Document Reference	BLT/LOC/00000/NEO

Aim: This document provides a rapid guide to the treatment of circulatory compromise in the newborn infant. Discussion of definitions and controversies takes place at the end of the guideline.

Recognition

Compromise of the neonatal circulation should be suspected in any infant who has hypotension, tachycardia or poor tissue perfusion. These are defined as follows:

1. **Hypotension - low mean arterial blood pressure.** At the Royal London Hospital this has a very simple definition, used throughout the first week of life:
   - <35mmHg in term infants and preterm infants ≥35 weeks
   - <30mmHg in preterm infants between 24 and 34 weeks gestation
   - <28mmHg in those 22-23 weeks gestation

   After one week, reduction from previously stable levels may indicate a problem
2. **Tachycardia** (HR>160/min in preterm, >140/min in term infants)
3. **Impaired tissue perfusion**, which may be indicated by:

- **Capillary refill time >3 seconds** (only limited usefulness in the neonate)
- **Lactate >2mmol/l** on accurate analyser, or **base excess <-8mmol/l** with normal chloride. Beware inborn errors of metabolism or post-asphyxial mitochondrial dysfunction, when the direction of change will be more useful than absolute values.
- A **low measured cardiac output** (LVO or RVO) of less than 150 ml/kg/minute. In the presence of an unrestrictive PDA with L→R shunt, the LVO may be considered low if it is <200ml/kg/min.
- **Poor organ blood flow** as measured by Doppler ultrasound or other appropriate method
- **ScVO2 < 70%** - oxygen saturation of less than 70% in blood sampled from the right atrium or from a UVC at the junction with the IVC
- **Persistent oliguria** <1ml/kg/hr, especially after 24 hours of age. Oliguria may be normal in the first day of life.

Measurement of blood pressure

Invasive monitoring via an arterial line is the gold standard and should ideally be used in all babies where circulatory compromise is suspected. Please note that cuff blood pressures may be markedly inaccurate.

Goals of therapy

Once treatment for circulatory compromise has commenced, it should be escalated until reversal of these indicators has been achieved. This will mean:

- Blood pressure above the intervention level
- Heart rate <180/minute
- Minimal or no evidence of impaired tissue perfusion using the above criteria
- Achieving the above without precipitating periventricular haemorrhage

Therapy should be maintained for a stable period of at least 4 hours, then carefully weaned.

Treatment for premature patients <35 weeks gestation in the first week of life

The accompanying flow diagram outlines the treatment protocol (see next page).

Treatment for patients >35 weeks gestation and preterm infants over one week of age

These patients will usually only develop circulatory compromise if they have serious underlying pathology. Treatment should be geared to these underlying problems:

- In septic shock and necrotising enterocolitis, very large volumes of fluid may need to be rapidly infused, of the order of 60-80 mls/kg
- In asphyxia or cardiomyopathy, large fluid volumes may overload a failing myocardium, consider echocardiography before giving volume
- Consider congenital heart disease in the differential diagnosis
- In PPHN, keep the MABP at 40-60mmHg
- In PPHN, consider early discussion with the ECMO service, especially once the oxygenation index is approaching 25 ($OI = (MAP \times \%FiO_2) / (PaO_2 \times 7.5)$) where MAP is airway pressure in cmH_20 and PaO_2 is in kPa
- Do not use indomethacin for PDA in the term baby
- Otherwise, follow the algorithm for premature infants

Management of Shock and hypotension in preterm infants <35 weeks gestation in the first week of life

Initial treatment commences with basic measures of adequate resuscitation, ventilation, establishment of vascular access. For all patients the aim is to achieve basic normalisation of oxygenation, acid-base status and blood glucose. Unless there is evidence of blood loss or hypovolaemia, rapid and large infusion of volume should be avoided as this may precipitate periventricular haemorrhage.

After initial treatment, the protocol uses different management according to whether there is hypotension, impaired tissue perfusion, or both of these factors. Following an initial time-limited phase, evaluation may lead to a number of different treatment pathways depending on the nature of the circulatory pathology.

	↓		
Time (min)	Labour room resuscitation and admission care - may include where appropriate surfactant administration, respiratory support, intravenous volume, blood, alkali, glucose or adrenaline		
30	Assessment on admission to neonatal unit		
15	Low tissue perfusion - as assessed by clinical criteria or measurement - normal BP	Both low MABP and low blood flow	Low MABP (defined as <30mmHg at 24-34 weeks gestation, sustained for more than 5 minutes)
45	**Ensure adequate SaO2, PaCO2, pH, lung expansion, glucose and calcium**		
60	10 to 20 mls/kg intravascular volume - this may include saline, blood, FFP or platelets (may be exceeded only if there is clear evidence of blood loss - APH, abruption, severe pallor, low PCV)		
60	Commence dobutamine 5 mcg/kg/min (min infusion rate 0.5 ml/hr)	Commence dopamine 5 mcg/kg/min (min infusion rate 0.5 ml/hr)	
30	Increase dobutamine 10 mcg/kg/min (min infusion rate 1.0 ml/hr)	Increase dopamine to 10 mcg/kg/min (min infusion rate 1.0 ml/hr)	
30	Commence dopamine 5 mcg/kg/min (min infusion rate 0.5 ml/hr)	Increase dopamine to 15 mcg/kg/min	
30	Increase dopamine to 10 mcg/kg/min	Increase dopamine to 20 mcg/kg/min	
	Evaluation of circulatory compromise - echocardiography, SVC flow, NIRS, ScvO2, or clinical assessment - also exclude pneumothorax, pulmonary overdistension or tamponade. Further treatment will depend on the nature of the circulatory disturbance – see next page		

Inotrope concentrations

Do not start inotrope infusions at less than 0.5 ml/hr as it will take too long for effective drug delivery to reach a stable state. For dopamine and dobutamine, use this formula:
Put 30mg/kg in a 50 ml syringe of 10 or 15 % dextrose
Start at infusion rate of 0.5 ml/hr which will give 5 mcg/kg/min

↓	↓	↓	↓	↓
Low intravascular volume or ventricular filling	Poor contractility, low RVO/LVO	High cardiac output	PPHN	PDA with unrestrictive L to R flow
20 mls/kg volume	Add Dobutamine 10 mcg/kg/min if not already given	Dopamine 20 mcg/kg/min, stop dobutamine	Pulmonary vasodilator preferably iNO 10-20ppm; combined with effective ventilation (may include HFOV); aim for MABP of 40mmHg (for CVS support see other categories - esp poor contractility & volume)	Indomethacin 0.1mg/kg (see other categories for augmentation of LVO if this is not already raised)
Re-evaluate, repeat if necessary	Adrenaline infusion at 50-500 ng/kg/min	Pressor infusion (Noradrenaline 50-500ng/kg/min or Adrenaline 50-500 ng/kg/min)	Epoprostenol infusion (5-40 ng/kg/min) and/or other systemic vasodilator such as IV magnesium sulphate (200 mg/kg then 20-50mg/kg/hr), IV adenosine or tolazoline (1-2mg/kg)	Re-evaluate after 12 hours, repeat if necessary
Hydrocortisone 2.5 mg/kg (2 doses 4 hours apart, then 6-hourly, halve dose every 48 hours once response seen; need for hydrocortisone may be supported by low plasma cortisol).			ECMO is likely to be contraindicated at this gestation but discussion with ECMO team may be appropriate	Re-evaluate after 24 hours, consider Indomethacin 0.2mg/kg
Re-evaluation of circulatory compromise - echocardiography, SVC flow, NIRS, ScvO2, or clinical assessment				
Re-evaluate, repeat if necessary	Infusion of Milrinone (either 0.75 mcg/kg/min for 3 h, then maintenance infusion of 0.2 mcg/kg/min ***or*** 0.3-1 mcg/kg/min) **or** Epoprostenol (5-40 ng/kg/min) & recheck pH, glucose, calcium	Higher doses of noradrenaline up to 1 mcg/kg/min	Milrinone infusion (either 0.75 mcg/kg/min for 3 h, then maintenance infusion of 0.2 mcg/kg/min ***or*** 0.3-1 mcg/kg/min) ***or*** sildenafil	PDA ligation is currently not used in this situation, but can be considered

↓

In refractory low-output states, drugs which may be considered experimental include milrinone (phosphodiesterase inhibitor with positive inotropic and vasodilator effects). Pentoxifilline is a phosphodiesterase inhibitor which may also improve the micro-circulation through increasing red cell deformability, but caution should be used in preterm neonates as its inhibition of platelet aggregation could predispose to haemorrhage (quoted dose regimes include 5mg/kg/hr over 6 hours (Ref 19)). Remember at all stages to ensure adequate pH, glucose and calcium levels.

CAUSES OF NEONATAL HYPOTENSION

(a) Low cardiac output

- Poor LV filling
  - Hypovolaemia
  - Poor venous return (e.g. overdistended lungs,tamponade)
  - Pulmonary hypertension
- Poor myocardial contractility
  - -asphyxia
  - -metabolic derangements (esp pH, Ca, glucose)
  - -sepsis
- Congenital heart disease
  - - Aortic stenosis
  - - hypoplastic LH
  - - cardiomyopathy

(b) Low distal vascular resistance

- ductal steal (PDA)
- low Peripheral VR

Controversies

Conditions related to circulatory disturbance, particularly in the preterm baby, remain an important cause of mortality and morbidity. These conditions include brain injury (periventricular haemorrhage and leucomalacia), gastrointestinal injury (necrotising enterocolitis), renal failure and septic shock. Some studies have demonstrated links between neonatal brain injury, low blood pressure (2) and low blood flow states (3) but these links remain a subject of debate.

Of the 2528 neonatal deaths in England and Wales in 2005, many are related to problems which could be amenable to support of the circulation (4). These include disorders related to short gestation and low birth weight (1055 deaths), respiratory and cardiovascular disorders originating in the perinatal period (93 deaths), bacterial sepsis of the newborn (102 deaths), intraventricular haemorrhage (over 100 deaths) and necrotising enterocolitis (68 deaths).

Systematic reviews (5) give guidance on the choice of drugs for first-line treatment of hypotension and low cardiac output, but not on the criteria for starting treatment, or for strategies to be used when first-line treatments fail. The most definite conclusion from these reviews is that "Dopamine improves low blood pressure in preterm babies more effectively than dobutamine in the short-term" (Subhedar & Shaw). Other reviews were insufficiently powered to detect effects for single interventions (such as adrenaline, dopamine, dobutamine or volume expansion compared with placebo). A more rewarding approach may be to introduce targeted packages of care within clear protocols.

Guidelines on treatment of shock have been used for adult and paediatric populations for several years. Once issued, these guidelines act as a service standard and as a stimulus to continuing research. Until recently, such guidelines have been lacking for the treatment of neonatal shock and hypotension. In older children with shock and hypotension, successful protocols are those with clear structures and timelines which individualise treatment to maximise organ integrity and perfusion, before irreparable damage occurs. In the setting of paediatric intensive care, introduction of these goal-directed systems of care have been associated with improved outcomes, particularly in meningococcal septicaemia (6,7).

In the care of newborn babies there is currently no agreed framework for the treatment of neonatal hypotension and shock, with less than 0.2% of the UK's major neonatal textbook (8) devoted to guidance on the treatment of hypotension. In this context, there is an increasing recognition of the need to individualise treatment using assessment of cardiac function (9).

Guidelines have been difficult to agree for newborn babies for a number of justifiable reasons. The neonatal circulation is in a state of transition from fetal to postnatal physiology. In the preterm, rapid volume expansion may be associated with periventricular haemorrhage (10). Even blood pressure varies considerably according to gestation and postnatal age. With the ethical issues associated with neonatal research and limited markets, commercial drug development for this population has been limited. As a consequence, many treatments are only tried in neonates after use in adult and paediatric populations.

There is debate over whether autoregulation of cerebral blood flow (CBF) can maintain constant flow to brain over a range of blood pressures in preterm infants. Studies using both Doppler and near infrared spectroscopy have shown loss of autoregulation in sick preterm infants and asphyxiated term infants(11-14). The degree of autoregulation to blood pressure may also be affected by medication (11,14). Munro et al showed that CBF correlated with mean arterial pressure (MAP) in hypotensive preterm infants and suggested a breakpoint of approximately 30 mmHg(15). Hypotensive preterm infants demonstrate little or no response in CBF to changes in arterial carbon dioxide, suggesting a complex interaction between BP, $PaCO_2$ and CBF(16).

General principles of calculating any infusion rate

A general formula to give an infusion where 1ml/hr = 1 mcg/kg/min
Put 3mg/kg in a 50 ml syringe
For higher infusion rates, multiply accordingly

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7.6 Treatment allocation form

An example of treatment allocation form for all three arms of the study for an infant recruited at 24 weeks gestation to the study.

Barts Health NHS
NHS Trust

Version 1_1/11/2012

Treatment Allocation Form

A pilot randomised trial comparing intervention levels for the support of blood pressure in extremely premature newborn babies

Hospital Number: Patient Identification Number for this trial:

Confirm that this baby is between 24^{+0} - 24^{+6} ☐

Gestation: 24 weeks

This baby has been allocated to the **'ACTIVE'** group.

Please record the baby's blood pressure hourly for the first 12 hourly and then 4 hourly for the first week of life.

Aim to support blood pressure if the mean arterial blood pressure falls **below 30 mmHg for 15 minutes.**

Weaning of inotropic support:

Blood pressure support will be escalated until the BP stabilises above 30 mmHg. Once the blood pressure is above this level, weaning will take place as follows:

1. If BP > 40 mmHg for 15 minutes, wean inotropic support immediately by at least 20% and continue to wean 1-2 hourly whilst BP stays at this level.
2. If BP > 35 mmHg , begin to wean after 6 hours of stability at this level and continue to wean 2-4 hourly whilst BP stays at this level.
3. If BP 31-35 mmHg, begin to wean after 12 hours of stability and continue to wean 2-4 hourly whilst BP stays at this level.

Barts Health NHS
NHS Trust

Version 1_1/11/2012

Treatment Allocation Form

A pilot randomised trial comparing intervention levels for the support of blood pressure in extremely premature newborn babies

Hospital Number: Patient Identification Number for this trial:

Confirm that this baby is between 24^{+0} - 24^{+6} ☐

Gestation: 24 weeks

This baby has been allocated to the **'MODERATE'** group.

Please record the baby's blood pressure hourly for the first 12 hourly and then 4 hourly for the first week of life.

Aim to support blood pressure if the mean arterial blood pressure falls **below 24 mmHg for 15 minutes.** Follow the unit guidelines for treatment of low blood pressure.

Weaning of inotropic support:

Blood pressure support will be escalated until the BP stabilises above 26 mmHg.

Once the blood pressure is above this level, weaning will take place as follows:

1. If BP > 34 mmHg for 15 minutes, wean inotropic support immediately by at least 20% and continue to wean 1-2 hourly whilst BP stays at this level.
2. If BP > 29 mmHg , begin to wean after 6 hours of stability at this level and continue to wean 2-4 hourly whilst BP stays at this level.
3. If BP >25 -29 mmHg, begin to wean after 12 hours of stability and continue to wean 2-4 hourly whilst BP stays at this level.

Barts Health NHS
NHS Trust

Version 1_1/11/2012

Treatment Allocation Form

A pilot randomised trial comparing intervention levels for the support of blood pressure in extremely premature newborn babies

Hospital Number: Patient Identification Number for this trial:

Confirm that this baby is between 24^{+0} - 24^{+6} ☐

Gestation: 24 weeks

This baby has been allocated to the **'PERMISSIVE'** group.

Please record the baby's blood pressure hourly for the first 12 hourly and then 4 hourly for the first week of life.

Aim to support blood pressure if there is clinical evidence of **poor tissue perfusion*** or if the mean arterial blood pressure falls **below 19 mmHg for 15 minutes.**

Weaning of inotropic support:

Blood pressure support will be escalated until the BP stabilises above 29 mmHg.

Once the blood pressure is above this level, weaning will take place as follows:

1. If BP > 29 mmHg for 15 minutes, wean inotropic support immediately by at least 20% and continue to wean 1-2 hourly whilst BP stays at this level.
2. If BP > 24 mmHg , begin to wean after 6 hours of stability at this level and continue to wean 2-4 hourly whilst BP stays at this level.
3. If BP 20-24 mmHg, begin to wean after 12 hours of stability and continue to wean 2-4 hourly whilst BP stays at this level.

❖ Including colour, heart rate, capillary refill time, urine output, worsening base excess and rising lactate.

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